Fructose reprograms glutamine-dependent oxidative metabolism to support LPS-induced inflammation

Nicholas Jones1,7, Julianna Blagih2,7, Fabio Zani2, April Rees1, David G. Hill3, Benjamin J. Jenkins1, Caroline J. Bull3,4, Diana Moreira5, Azari I. M. Bantan1, James G. Cronin1, Daniele Avancini6, Gareth W. Jones3, David K. Finlay5, Karen H. Vousden2, Emma E. Vincent3,4,8 & Catherine A. Thornton1,8

Fructose intake has increased substantially throughout the developed world and is associated with obesity, type 2 diabetes and non-alcoholic fatty liver disease. Currently, our understanding of the metabolic and mechanistic implications for immune cells, such as monocytes and macrophages, exposed to elevated levels of dietary fructose is limited. Here, we show that fructose reprograms cellular metabolic pathways to favour glutaminolysis and oxidative metabolism, which are required to support increased inflammatory cytokine production in both LPS-treated human monocytes and mouse macrophages. A fructose-dependent increase in mTORC1 activity drives translation of pro-inflammatory cytokines in response to LPS. LPS-stimulated monocytes treated with fructose rely heavily on oxidative metabolism and have reduced flexibility in response to both glycolytic and mitochondrial inhibition, suggesting glycolysis and oxidative metabolism are inextricably coupled in these cells. The physiological implications of fructose exposure are demonstrated in a model of LPS-induced systemic inflammation, with mice exposed to fructose having increased levels of circulating IL-1β after LPS challenge. Taken together, our work underpins a pro-inflammatory role for dietary fructose in LPS-stimulated mononuclear phagocytes which occurs at the expense of metabolic flexibility.

1 Institute of Life Science, Swansea University Medical School, Swansea University, Swansea, UK. 2 The Francis Crick Institute, London, UK. 3 Cellular and Molecular Medicine, University of Bristol, Bristol, UK. 4 MRC Integrative Epidemiology Unit, University of Bristol, Bristol, UK. 5 School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland. 6 San Raffaele Telethon Institute for Gene Therapy, San Raffaele Scientific Institute, Milan, Italy. 7 These authors contributed equally: Nicholas Jones, Julianna Blagih. 8 These authors jointly supervised this work: Emma E. Vincent, Catherine A. Thornton. ✉ Email: emma.vincent@bristol.ac.uk; c.a.thornton@swansea.ac.uk
Typically, activation of the human innate immune system requires the rewiring of cellular metabolic pathways largely to favour glucose metabolism. However, in the various nutrient environments they inhabit, monocytes will be exposed to a range of different carbon sources, the availability of which will likely dictate their metabolism and phenotype. One such carbon source is fructose, the second most abundant dietary sugar found in humans. Fructose is metabolised by glycolysis either by keto-hexokinase producing fructose-1-phosphate, a substrate for aldolase B (in the liver, kidneys and intestines for example) or converted to the glycolytic intermediate fructose-6-phosphate by hexokinase (HK), albeit at a lower rate than glucose.

Fructose intake has increased substantially throughout the Western world, largely attributed to elevated sucrose and high fructose corn syrup consumption and is thought to exacerbate various non-communicable conditions such as obesity, type 2 diabetes and non-alcoholic fatty liver disease. Chronic fructose consumption in these conditions has recently been shown to drive hepatic fructolysis, where the expression of lipogenic genes is enhanced.

Typically, physiological levels of fructose in the circulation range from 0.04 to 0.2 mM, however, there are several pathological scenarios in which levels of fructose are elevated. For example, peripheral blood levels can exceed 1 mM in patients with haematological malignancies such as acute myeloid leukaemia and acute lymphoblastic leukaemia. In addition, fructose concentrations in the bone marrow microenvironment of haematological cancer patients can reach up to 5 mM. Alterations in the glucose to fructose ratio, particularly when glucose is scarce, enables acute myeloid leukaemia blasts to significantly enhance fructose uptake. Localised mouse tissue microenvironments, such as the liver, kidneys and jejunum, also have elevated levels of fructose metabolism. Therefore, there are various pathophysiological scenarios and tissue microenvironments where monocytes will be exposed to either equimolar concentrations of fructose and glucose or concentrations of fructose exceeding that of glucose.

The impact of elevated fructose exposure on the immune system has not been investigated extensively. Chronic fructose exposure in rats results in a more inflammatory phenotype of bone marrow mononuclear cells. While there is some evidence that lipopolysaccharide (LPS)-stimulated human dendritic cells are able to produce enhanced levels of pro-inflammatory cytokines when cultured in fructose as opposed to glucose, the underlying metabolic rewiring that enables this pro-inflammatory phenotype has not been investigated.

Here we characterise how human monocytes and mouse macrophages respond metabolically and functionally to fructose exposure. We show that activated mononuclear phagocytes demonstrate plasticity in engaging metabolism of this alternative carbon source, yet it leaves the cells metabolically inflexible and vulnerable to further metabolic challenge. Fructose exposure reprogrammes cellular pathways to favour glutaminolysis and oxidative metabolism, which support an inflammatory phenotype in both human and mouse mononuclear phagocytes. Finally, we demonstrate that a short-term high fructose diet promotes inflammation in vivo, suggesting that our findings have pathophysiological significance.

**Results**

**Fructose exposure promotes an oxidative phenotype.** We sought to investigate the metabolic response to fructose exposure in activated human monocytes in comparison to other monosaccharides (glucose and galactose) or complete sugar withdrawal. Galactose has been used previously to promote oxidative phosphorylation (OXPHOS) in T cells by reducing glycolysis. Using the Seahorse Bioanalyzer, we initially injected the monosaccharide (glucose, fructose or galactose) or media control containing no sugar. After 1 h, monocytes were stimulated with LPS and the corresponding extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were used to measure glycolysis and OXPHOS, respectively, for the duration.

Monocytes incubated with glucose demonstrated a robust increase in basal and LPS-induced ECAR (Fig. 1A). By contrast, monocytes treated with fructose or galactose had low baseline levels of glycolysis, only slightly greater than no sugar controls (Fig. 1A). In addition, monocytes treated with fructose, galactose or no sugar had only a modest increase in ECAR post-LPS exposure (Fig. 1A). Upon activation, glucose-treated cells reduced their OCR, consistent with the reported metabolic switch from OXPHOS to glycolysis upon activation. Monocytes treated with fructose, galactose or no sugar had an initial burst of increased oxygen consumption and maintained higher OCR for the duration of the assay (Fig. 1B). This demonstrates metabolic flexibility of human monocytes, in this case towards OXPHOS when the cells are unable to engage in glycolysis.

After the introduction of 2-deoxy-D-glucose (2-DG), ECAR was reduced in all conditions (Fig. 1C). OCR was increased in glucose-treated monocytes, a compensatory response to glycolysis inhibition, whereas in fructose, galactose or no sugar treatment a decrease in OCR was observed (Fig. 1D). These data suggest that LPS-stimulated monocytes treated with fructose or galactose direct pyruvate towards the mitochondrial tricarboxylic acid (TCA) cycle for OXPHOS, whereas glucose treatment directs pyruvate towards lactate production. We confirmed extracellular levels of lactate are indeed reduced in fructose compared to glucose-treated monocytes (Supplementary Fig. 1A). This divergence appears to account for the differences in OXPHOS rates observed. Overall, LPS-stimulated human monocytes treated with fructose maintained an elevated oxidative phenotype with low ECAR, akin to galactose or no sugar treatment, whereas glucose availability preferentially maintained elevated levels of glycolysis at the expense of reduced oxygen consumption (Fig. 1E).

In agreement with a previous study, LPS-stimulated monocytes cultured for 24 h with galactose or no sugar had a significant reduction in viability in comparison to the glucose-treated control (Fig. 1F). However, we observed no difference in viability between glucose and fructose treatment (Fig. 1F).

To further elaborate fructose metabolism in LPS-stimulated monocytes, we incubated them with either uniformly labelled $^{13}$C$_6$-fructose or $^{13}$C$_6$-glucose and performed stable isotope tracer analysis (SITA). Activated monocytes were able to transport fructose into the cell (presumably via GLUT5 expression—Supplementary Fig. 1B) and incorporated comparable levels of carbon into intracellular lactate while increasing incorporation into TCA cycle intermediates and amino acids in comparison to glucose (Supplementary Fig. 1C–E). These results suggest that the cells have the ability to metabolise fructose carbon and use it in the TCA cycle.

Collectively, these data reveal that fructose treatment promotes a low glycolytic rate without compromising cell viability. This demonstrates the metabolic flexibility of human monocytes as well as their ability to utilise an alternative carbon source.
bioenergetic changes were monitored over time. While OCR of both glucose or fructose LPS-simulated monocytes decreased to the same level upon oligomycin treatment (Fig. 2A), the drastic reduction of OCR in fructose-cultured monocytes reflects a greater reliance on OXPHOS. Cells reliant on OXPHOS may demonstrate metabolic flexibility upon oligomycin treatment by increasing glycolysis. However, surprisingly, ECAR in fructose-cultured monocytes decreased in response to oligomycin, suggesting the lack of metabolic adaptation in these cells (Fig. 2B, C).

Secondly, to establish whether the elevated ECAR levels post-LPS treatment reflected glycolytic activity as opposed to other acidifying processes, we used a lactate dehydrogenase inhibitor (GSK2837808A; LDHi). Here, the increased ECAR in response to LPS stimulation was reduced in glucose-treated monocytes upon LDHi treatment (Fig. 2D). By contrast, LDHi modestly impacted ECAR in fructose-treated cells, arguing that fructose-mediated glycolysis is coupled to OXPHOS. The low level of ECAR under this condition is most likely due to acidification of the media by an alternative source to lactate (Fig. 2D). We confirmed this was not due to changes in LDH phosphorylation in fructose- versus glucose-treated cells (Supplementary Fig. 1F).

The corresponding OCR levels increased in the glucose-treated monocytes upon LDHi treatment as pyruvate is directed towards OXPHOS, again demonstrating the bioenergetic flexibility of human monocytes. By contrast, OCR in fructose-treated monocytes remained unchanged for the duration of the assay (Fig. 2C, F).

Fructose treatment enhances LPS-induced inflammation. Given the distinct metabolic characteristics of human monocytes exposed to fructose (Fig. 1A), we were intrigued to investigate the impact on monocyte function. Fructose-treated monocytes produced elevated levels of a panel of secreted cytokines, namely interleukin-1β (IL-1β), IL-6, IL-8, IL-10 and tumour necrosis factor (TNF) (Fig. 3A–E), with IL-1β, IL-8, IL-10 and TNF reaching statistical significance in comparison to glucose treatment. These data further demonstrate that glycolysis and OXPHOS are tightly coupled in fructose-treated cells, revealing impaired metabolic flexibility in comparison to glucose-treated cells (Fig. 2C, F).
RNA-sequencing (RNA-seq) analysis of LPS-stimulated monocytes treated with glucose or fructose. Surprisingly, we observed only five genes that were significantly changed between the two conditions, with no alteration to transcript levels for the cytokines of interest (Supplementary Fig. 2B). Consistent with this, the expression level of genes encoding cytokines was also comparable in monocytes treated with either glucose or fructose (Fig. 3F), suggesting that the increased cytokine production was not through transcriptional regulation. Fructose has recently been reported to activate mTORC1 via dihydroxyacetone phosphate sensing20. Consistent with this, phosphorylation of the downstream mTORC1 target, S6 ribosomal protein, was elevated significantly in LPS-stimulated monocytes treated with fructose (Fig. 3G). Of note, we observed no differences in the induction of AMPK (AMP-activated protein kinase) signalling between the two groups (Supplementary Fig. 2C). Collectively, these observations demonstrate that LPS stimulation in the presence of fructose enhances inflammatory cytokine production, in part, through increased mTORC1-mediated translation.

![Figure 2](https://example.com/fig2.png)

**Fig. 2 Fructose treatment enhances oxidative metabolism.** Seahorse extracellular flux analysis of A OCR and B ECAR in monocytes before and following injections of glucose or fructose (both 11.1 mM), LPS (10 ng/ml) and oligomycin (1 µM) at the time points indicated. C OCR versus ECAR map of average of a single value pre- and post-oligomycin injection for glucose- and fructose-treated monocytes. Arrows indicate the shift in metabolism upon inhibitor exposure. Analysis of D ECAR and E OCR in monocytes as A with a final injection of a lactate dehydrogenase inhibitor (GSK2837808A, LDHi; 10 µM). F OCR versus ECAR map as C with pre- and post- LDHi injection. Data are representative of three independent experiments and are expressed as mean ± SEM. Source data are provided as a Source Data file.

Fructose treatment drives a sustained oxidative phenotype. LPS-stimulated monocytes in the presence of fructose clearly enhance oxygen consumption in the short term (Fig. 1C). Next, we wanted to determine whether heightened oxygen consumption was sustained long term in activated monocytes. Here, using the Seahorse Bioanalyzer, we performed a mitochondrial stress test with a final injection of the ionophore, monensin21, following a 24-h incubation period with either glucose or fructose. Elevated levels of oxygen consumption were indeed sustained long term in fructose-treated LPS-stimulated monocytes (Fig. 4A). This was characterised by increased levels of oxidative parameters such as basal respiration, ATP-linked respiration and a higher percentage of coupling efficiency in comparison to glucose (Fig. 4B–D and Supplementary Fig. 3A–D). Corresponding ECAR levels were unsurprisingly higher in glucose-treated monocytes in comparison to fructose-treated cells (Fig. 4E, F and Supplementary Fig. 3E). Notably, fructose-treated monocytes had a significantly higher ratio of basal OCR/ECAR ratio in comparison to glucose, indicating their commitment to oxidative metabolism (Fig. 4G).

We next assessed the contributions of glycolysis derived- and OXPHOS-derived ATP production21. These analyses show glucose-treated monocytes as glycolytic cells functioning at their maximal glycolytic ATP production rate at baseline (Fig. 4H). At maximal bioenergetic capacity, they demonstrate flexibility towards ATP production from OXPHOS, but still derive the majority of their ATP from glycolysis and therefore remain classed as glycolytic cells (Fig. 4H and Supplementary Fig. 3F, G). By contrast, fructose-treated monocytes are oxidative (deriving the majority of their ATP from OXPHOS) at baseline and this phenotype is further exacerbated when cells are at maximal bioenergetic capacity (Fig. 4H and Supplementary Fig. 3F, G). They do, however, have some metabolic scope to increase ATP production from glycolysis (Fig. 4H).

We reasoned that differential mitochondrial properties might explain the heightened oxidative phenotype of fructose-treated monocytes. However, we observed no difference in mitochondrial content (MitoTracker Green), membrane potential (tetramethylrhodamine ethyl ester) or mitochondrial-derived reactive oxygen species (ROS) (Fig. 4I). Further to this, we also assessed levels of the individual respiratory complexes by immunoblot. Again, we observed no difference in the abundance of complexes I–IV between fructose- or glucose-treated monocytes (Fig. 4J). A high fructose diet has been shown to increase de novo lipogenesis in the liver10. This correlates with increased mitochondrial ATP production, which may support this energy-demanding process22,23.
Therefore, a potential explanation for the elevated ATP-linked respiration observed is that fructose-treated monocytes are supporting a higher level of lipogenesis. We observed no differences in phosphorylation of enzymes that catalyse the citrate-derived fatty acid synthesis steps; ATP citrate lyase (ACLY) or acetyl-CoA carboxylase (Supplementary Fig. 3H). However, fructose-cultured, LPS-stimulated monocytes have increased levels of the lipid mediator, prostaglandin E2 and greater sensitivity to the ACLY inhibitor, BMS303141, with regards to cytokine production (Supplementary Fig. 3I, J). Taken together, these data demonstrate that fructose treatment elevates ATP-linked oxygen consumption, independent of mitochondrial ROS and content.

Fructose increases TCA cycling and anaplerosis. To further characterise the metabolic activity of the mitochondria in glucose- and fructose-treated cells, we used SITA coupled with gas chromatography-mass spectrometry (GC-MS). Here, human monocytes were activated with LPS for 24 h and incubated with either $^{13}$C$_6$-glucose or $^{13}$C$_6$-fructose (Fig. 5A). Mass isotopologue distribution (MID) analysis of the TCA cycle intermediates—succinate, fumarate and malate—and amino acids—glutamate and aspartate—highlighted that there was increased cycling in the fructose-cultured monocytes compared to glucose. This was indicated by a reduced proportion of the unlabelled form of the metabolite ($m+0$) and an increased proportion of the labelled form (predominantly represented by $m+2$) (Fig. 5B, C and Supplementary Fig. 4A).

The TCA cycle relies on other metabolites to replenish it, a process termed anaplerosis. Glutamine-derived carbon enters the TCA cycle and also contributes to TCA-mediated amino acid biosynthesis, such as aspartate. To investigate whether fructose treatment increases glutamine anaplerosis, we performed SITA with $^{13}$C$_5$-glutamine. Here, monocytes were incubated with $^{13}$C$_5$-glutamine in the presence of either glucose or fructose (Supplementary Fig. 4C-G). These data demonstrate that fructose treatment increases the proportion of both sugar and glutamine carbon into the TCA cycle to support the observed increased rates of OXPHOS.

Fructose-treated human monocytes are vulnerable to metabolic challenge. Thus far, we have demonstrated that fructose treatment promotes a reduced glycolytic rate and enhanced OXPHOS. Glucose and fructose are both metabolised by the enzyme HK, producing metabolites glucose 6-phosphate or fructose-6-phosphate respectively. Therefore, we sought to investigate the expression levels of the two predominant isoforms of HK: HKI and HKII. We observed no difference in expression levels of HKI; however, HKII levels were increased in fructose-treated, LPS-stimulated monocytes, in comparison to glucose (Fig. 6A, B). To further delineate the role of HK in monocyte function, we utilised the HK inhibitor, 2-DG. We treated activated monocytes in glucose or fructose for 24 h with 2-DG before measuring cytokine production. Following treatment with 2-DG, production of IL-1$, \beta$, IL-6 and TNF was largely unaffected in glucose-treated cells, with the exception of IL-10. By contrast, fructose-treated...
cells exhibited a dose-dependent decrease in all cytokines (Fig. 6C).

Having shown that 2-DG treatment reduced OXPHOS in fructose-treated cells (Fig. 1D), these results suggest the oxidative metabolic phenotype induced by fructose supports monocyte function. Given the enhanced reduction of cytokine expression, we next determined the level of cell viability for 2-DG-treated monocytes. Fructose-treated monocytes were acutely sensitive to 2-DG-mediated cell death in comparison to glucose-treated monocytes that were largely unaffected (Fig. 6D). We next wanted to assess whether the cells were similarly sensitive to inhibition of oxidative metabolism. The viability of LPS-stimulated monocytes treated with glucose was minimally affected by inhibition of complex I (with rotenone), III (with antimycin A) and V (with oligomycin). However, monocytes treated with fructose were completely unable to tolerate incubation with these drugs, reflected by a striking reduction in viability (Fig. 6E). These data demonstrate that fructose treatment renders LPS-stimulated monocytes metabolically inflexible and dependent on oxidative metabolism. Sensitivity to both 2-DG and mitochondrial inhibitors suggests that glycolysis and oxidative metabolism become inextricably coupled upon exposure to fructose.

Fig. 4 Fructose-induced oxidative phenotype is maintained in LPS-stimulated monocytes. A Mitochondrial stress assay of monocytes cultured for 24 h in the presence of glucose or fructose (both 11.1 mM) and activated with LPS (10 ng/mL). Corresponding OCR measured with injections of oligomycin (1 µM), FCCP (3 µM), antimycin A (1 µM) and rotenone (1 µM) and monensin (20 µM). Respective mitochondrial parameters B basal respiration, C ATP-linked respiration and D coupling efficiency calculated via ATP-linked respiration/basal respiration. E Equivalent ECAR rate measured with F basal glycolysis levels. G OCR/ECAR ratio calculated by basal respiration/basal glycolysis levels. H Bioenergetic scope examining the ATP production ($\text{J}_{\text{ATP}}$) of oxidative phosphorylation versus glycolysis of basal and maximal of glucose or fructose. Mitochondrial parameters I Content: MitoTracker Green (MTG), membrane potential: tetramethylrhodamine ethyl ester (TMRE) and mitochondrial-derived ROS: MitoSOX measured by flow cytometry. J Mitochondrial respiratory complex (I-V) assessment by western blot. Statistical significance was assessed using an unpaired, two-tailed t test (B–D, F–B). Data are representative of four (A–H, J) or three (I) independent experiments. Data are expressed as mean ± SEM. Source data are provided as a Source Data file.
Dietary fructose increases inflammation in a mouse model. Thus far, we have explored the metabolic and mechanistic implications of fructose exposure in human monocytes cultured ex vivo. In order to further investigate the impact of physiological fructose exposure on inflammation in vivo, we employed a mouse model. First, we determined whether mouse LPS-challenged bone marrow-derived macrophages (BMDMs) phenocopied human monocytes when exposed to fructose in vitro.

To better recapitulate the in vivo microenvironment, we incubated mouse macrophages with either glucose alone or a 1:1 ratio of glucose to fructose (maintaining the equivalent concentration of total monosaccharide). This strategy has been used previously in several studies to investigate the physiological impact of fructose exposure in an environment when glucose is also present. Consistent with human monocytes, LPS-stimulated mouse macrophages exposed to fructose produced elevated levels of cytokines TNF, IL-1β, IL-6 and IL-12 at the protein level, but not the messenger RNA level in comparison to glucose alone (Fig. 7A and Supplementary Fig. 5A, B). Importantly, we demonstrated that this observation is not due to reduced glucose availability in the double monosaccharide condition (Supplementary Fig. 5C).

In order to confirm fructose uptake in the presence of glucose, we performed SITA analysis using mouse macrophages cultured in universally labelled $^{13}$C$_6$-glucose alone or $^{13}$C$_6$-glucose with $^{13}$C$_1$-fructose (Fig. 7B). Indeed, fructose enters the cells in the presence of glucose as depicted by the presence of the m + 1 isotopologue (Fig. 7C). Similar to human monocytes, BMDMs exposed to fructose significantly increased glutamine uptake and phosphorylation of the mTORC1 target, S6 ribosomal protein (Fig. 7D, E). Fructose treatment also led to an increase in Akt phosphorylation (Supplementary Fig. 5D).

Next, we investigated the role of glutamine metabolism in fructose-treated mouse macrophages and their response to LPS. The glutaminase inhibitor CB-839 did not alter levels of TNF; however, it significantly reduced IL-1β and IL-12 in BMDMs cultured in the presence of both monosaccharides, whereas cytokine production was unchanged in cells cultured with glucose alone (Fig. 7F). CB-839 also reduced phosphorylation of S6 in fructose-exposed cells (Supplementary Fig. 5E), suggesting that increased glutamine metabolism supports mTORC1 activity in the presence of fructose. Further exploring the role of mTORC1 in fructose-mediated inflammation, we treated BMDMs with the mTORC1 inhibitor, rapamycin. Here, rapamycin treatment significantly reduced cytokine production in both glucose-alone and glucose–fructose-treated macrophages (Fig. 7G). These data suggest that mouse macrophages require mTORC1 activity for cytokine production regardless of sugar exposure, yet those exposed to both monosaccharides (in contrast to those exposed to glucose alone) rely on glutaminolysis to support the increased cytokine production promoted by fructose exposure.

Finally, to determine if fructose supplementation can influence LPS-induced inflammation in vivo, we used a mouse LPS model of systemic inflammation. Here, mice were provided 10% glucose or 10% fructose/10% glucose solutions for 2 weeks (to preclude development of any metabolic disorders) prior to LPS challenge (Fig. 7H). Strikingly, serum IL-1β levels were significantly increased in mice exposed to fructose, and an increasing trend of IL-6 and TNF was observed (Fig. 7I). This increase in serum IL-1β was not due to fructose-enhancing baseline IL-1β secretion, as seen at weeks 1 and 4 of sugar-water treatment in unchallenged mice (Supplementary Fig. 6A, B).

Interestingly, the presence of fructose does not result in a global increase in serum inflammatory markers, as while levels of CXCL5 were elevated in the presence of fructose, this did not reach statistical significance and there was no change in CXCL1 and CCL11 (Supplementary Fig. 6C–E). These data demonstrate that short-term fructose supplementation enhances LPS-induced systemic inflammation, suggesting physiological repercussions of high fructose exposure in mammals.

**Fructose-supported inflammation does not occur in T cells.** Given our results of an effect of fructose on both human and mouse mononuclear phagocytes, we were intrigued as to whether fructose exposure might have an effect on other immune cell types. However, functional observations of heightened...
Fig. 6 Fructose-induced inflammatory monocytes are sensitive to metabolic inhibition. Immunoblot of LPS-stimulated monocytes in the presence of glucose or fructose with A hexokinase I or B hexokinase II analysed. C Cytokine release (% of control) of IL-1β, IL-6, IL-10 and TNF (dotted line indicates control) and D representative flow cytometry plot including DRAQ7 viability of glucose or fructose LPS-stimulated monocytes in the presence or absence of glycolytic inhibitor, 2-DG (0.1 or 1.0 mM). E Representative flow cytometry plot including DRAQ7 viability of glucose or fructose LPS-stimulated monocytes in the presence or absence of complex I inhibitor, rotenone (1 µM), complex III inhibitor antimycin A (1 µM) or complex V inhibitor, oligomycin (1 µM). Statistical significance was assessed using an unpaired, two-tailed t test (A, B) or a two-way ANOVA with Sidak’s multiple comparison test (C, D). Data are representative of five (A), four (B), three-four (C), three (D) or two independent experiments (E) and are expressed as mean ± SEM; *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001. Source data are provided as a Source Data file.
inflammation were not reflected in the mouse T cell compartment. We did not observe any differences in CD4+ or CD8+ T cell proliferation (Supplementary Fig. 7A). CD4+ T cells can be programmed into inflammatory subsets, such as T-helper type 1 (Th1) or anti-inflammatory subsets such as inducible regulatory T cells. Fructose supplementation did not influence polarisation of either Th1 or inducible regulatory T cells (Supplementary Fig. 7B, C). This suggests that fructose has a specific effect on LPS-stimulated mononuclear phagocytes.

Discussion
Here, we investigate how activated human monocytes and mouse macrophages respond metabolically and functionally to fructose exposure. We demonstrate that mononuclear phagocytes from both species are metabolically plastic in engaging in the metabolism of an alternative carbon source and reprogramme cellular pathways to favour oxidative metabolism. Although able to rewrite their metabolic pathways upon exposure to fructose, the cells are left metabolically inflexible and vulnerable to further metabolic
challenge. Importantly, we show that fructose exposure ex vivo promotes elevated cytokine production in both human and mouse mononuclear phagocytes and that a high fructose diet promotes an inflammatory phenotype in vivo, attributing pathophysiological relevance to our findings.

To date, most research has focused on the role of the disaccharide sucrose (glucose and fructose) in macrophage function. Sucrose stimulates pinocytosis in unstimulated macrophages and is broken down in the lysosome. The only link to date between fructose and macrophage-mediated inflammation is that supra-physiological concentrations of sucrose (300 mM) enhance IL-1β secretion, most likely due to hypertonicity. There is some evidence that fructose enhances myeloid function, either by increasing cytokine production in human dendritic cells or phagocytes in mouse alveolar macrophages; however, the metabolic mechanisms of fructose-mediated inflammation have not been explored. Coupled with this is a growing concern regarding high fructose corn syrup consumption generally—in some cases, accounting for 10% caloric intake in the USA—thus under-scoring the need to understand the implications of fructose exposure on macrophage function and inflammation.

Here, for the first time, we show that LPS-stimulated human monocytic and mouse macrophages exposed to fructose have an enhanced inflammatory phenotype supported by oxidative metabolism and glutaminolysis. We suggest this supports cytokine production through increased supply of biosynthetic intermediates. Mechanistically, we demonstrate that fructose-exposed cells have increased mTORC1 activity, and while this is required to support cytokine production regardless of sugar exposure, those cells exposed to fructose rely specifically on glutaminolysis to support their inflammatory phenotype (Fig. 7). mTORC1 has previously been implicated in metabolic reprogramming in monocyes, with monocyte-specific deletion of Raptor (an mTORC1 scaffolding protein) leading to reduced oxidative metabolism during monocyte differentiation. mTORC1 has also been shown to be activated by glutaminolysis and α-ketoglutarate production. This is consistent with our findings and further suggests that mTORC1 can act both upstream and downstream of metabolic reprogramming.

We demonstrate that fructose-dependent metabolic reprogramming is maintained long term, but this is independent of changes to mitochondrial dynamics. Instead, it appears due to increased metabolic intermediate supply. Our data show that fructose carbon can contribute to both glycolysis and the TCA cycle, and whether this is mediated by HK or ketohexokinase in both human and mouse mononuclear phagocytes requires further exploration. In comparison to glucose, fructose-derived pyruvate is not converted to lactate. This could be due to reduced activity or expression of LDH (although our data would suggest otherwise), increased activity of pyruvate dehydrogenase and mitochondrial pyruvate carrier 1 or decreased activity of pyruvate dehydrogenase kinase 3, 33. Fructose exposure has also been linked extensively to lipid biosynthesis, for example, fructose as a substrate is 30% more efficient at synthesising fatty acids than glucose, a phenomenon that has been implicated in the pathophysiology of non-alcoholic fatty liver disease. Consistent with this, cytokine production was more sensitive to ACLY inhibition and levels of prostaglandin E2 were elevated in our fructose-treated monocyes.

It is clear that fructose levels fluctuate throughout health and disease. With the increased prevalence of high fructose diets in the Western world, understanding the impact of fructose on human health is critical. Fructose contributes to numerous metabolic disorders such as obesity, cancer and non-alcoholic fatty liver disease; however, to date, our understanding of its impact on the immune system is lacking. A key strength of our study is the assessment of the impact of fructose on metabolism and inflammation across the two species. Akin to human monocyes, mouse macrophages increased consumption of glutamine, produced higher levels of cytokine production displayed and demonstrated elevated mTORC1 activity. A mouse model of LPS-induced inflammation allowed us to assess the physiological relevance of our findings. Previous in vivo studies of long-term high fructose exposure have been in the context of metabolic disorders such as steatosis and hyperglycaemia. Here, in order to circumvent any changes due to whole-body metabolism, we used a 2-week exposure strategy. This allowed us to demonstrate for the first time that fructose enhances inflammation independent of metabolic disease. Collectively, this provides direct evidence that fructose elevates inflammation under physiological conditions.
in shaping the innate immune response and could form the foun-
dations of investigations for therapies in areas as diverse as cancer and infectious diseases.

**Methods**

**Human monocyte isolation and culture.** Peripheral blood was collected with informed written consent and ethical approval was obtained from Wales Research Ethics Committee 6 (13/WA/0190). Mononuclear cells were obtained by density gradient centrifugation (Histopaque-1077; Merck). Human CD14+ monocytes were isolated using CD14 microbeads (130-050-201; Miltenyi Biotec). The purity of monocytes was routinely monitored using anti-CD14 Pacific Blue (clone 63F3; 367122; BioLegend) via flow cytometry.

Monocytes (1.0 × 10^6/mL) were activated with LPS (10 ng/mL; Invivogen) and cultured in glucose (F3510; 11.1 mM; Merck) containing glucose-free RPMI (11879020; Thermo Fisher) supplemented with 25 ng/mL of macrophage colony-stimulating factor (315-02; PeproTech). Cells were harvested and analysed for flow cytometry (acquired with FlowJo V10.3). Mouse serum samples were analysed using ELISA for IL-1β (Sigma, RAB0274), TNF (Mouse Uncoated ELISA Kit; Invitrogen, 88-7324-22), IL-6 (Mouse Uncoated ELISA Kit; Invitrogen, 88-7064-22), CXCL1 (Raybiotech, ELM-KC-1), CXCL5 (Legends MAX, BioLegend, 443907). ELISA plates were coated with the capture antibody and left overnight at 4 °C. Samples were appropriately diluted and incubated for 2 h at room temperature with gentle agitation, 2 h with the kit-specific secondary antibody and 20 min with streptavidin-horseradish peroxidase. The plate was then incubated at room temperature with a 1:1 mixture of hydrogen peroxide and tetramethylbenzidine. Absorbance was then measured at 450 nm following the addition of a stop solution (Sigma, Stopping Solution). The absorbance values were calculated according to Pfaff et al. (1994).

**Antibodies.** Fluorescence-conjugated PerCP-Cy5.5 F4/80 (clone BM8; 121328), PE-CD14+ (clone MEM-30; 555014), anti-TNF, anti-IL-6 and pro-IL-1β were purchased from BD. The ACLY inhibitor, CB-839 (Merck, AMBH2D6FB23B) (1 µM per well) or rapamycin (R8781; Sigma) was added at the start of the experiment. Alternatively, monocytes were given a third injection of oligomycin (1 µM), FCCP (3 µM), antimycin A (1 µM) and monensin (20 µM; MP Biomedicals). Data were acquired using the Seahorse Wave software v2.6 (Agilent Technologies)

**Enzyme-linked immunosorbent assay.** Whole serum was collected with heparin (103578-100; Agilent Technologies). Monocytes (1.0 × 10^6 cells) were seeded onto a Cell-Tak (354240; Corning)-coated microplate along with poly-L-lysine (0.1%, Sigma) and anti-CD62L (clone DREG-56; 304806; BioLegend), anti-CCR5 (clone REA245; 130-117-356) and anti-CCR2 (clone REA624; 130-113-283) and anti-CD80 (clone REA661; 130-110-270), anti-CD86 (clone FM95; 130-113-095; all Miltenyi Biotec) and anti-CD14 (clone 63F3; 367122; BioLegend) via flow cytometry.

**Flow cytometry and intracellular cytokine staining.** Human monocytes. Flow cytometry analysis was performed on monocytes after 24 h in culture. Cell death was monitored with DRAQ7 (1 µM, DR71000; BioStatus) and DRAQ7 negatives were analysed with anti-HLA-DR (clone AC22C; 130-095-293), anti-CD80 (clone REA661; 130-110-270), anti-CD86 (clone FM95; 130-113-572), anti-CCR5 (clone REA245; 130-117-356) and anti-CCR2 (clone REA624; 130-109-395; all Miltenyi Biotec) and anti-CD62L (clone DREG-56; 304806; BioLegend).

**Mouse BMDM differentiation and culture.** Hind legs were collected from C57/B6 mice (aged 6–15 weeks) and supernatants were stored at −20 °C for cytokine analysis.

**Mice.** Animal experiments were subject to ethical review by the Francis Crick Animal Welfare and Ethical Review Body and regulation by the UK Home Office project licence P319A£988. All mice were housed under conditions in line with the Home Office guidelines (UK). Mice were housed 3–5 per cage and were kept in a 12-h day/night cycle 07:00–19:00. Food and water were available ad libitum and rooms were kept at 21 ± 5% humidity. All procedures were performed following the Animals (scientific procedures) Act 1986 and the EU Directive 2010/63/EU.

**Staining and flow cytometry.** Cells were fixed and stained as recommended in the TruSeq Stranded Total RNA Kit (Illumina Inc.). The steps included ribosomal RNA depletion and cleanup, RNA fragmentation, adapter ligation and PCR amplification.

**Extracellular lactate measurement.** Extracellular lactate was measured using the l-Lactate Assay Kit (I20001100A; Eton Biosciences). Samples and standards were appropriately diluted and mixed with the l-lactate assay solution and incubated at 37 °C for 30 min. Absorbance was then measured at 490 nm and concentrations calculated from the linear regression of the standard curve.

**RNA extraction and quantitative PCR of BMDMs.** Total RNA was extracted using RNeasy® columns (Qiagen) from three separate wells per condition according to the manufacturer’s instructions. Genomic DNA was removed using On-Column DNA digestion (Qiagen). Complementary DNA (cDNA) was generated using the High-Capacity cDNA Reverse Transcription Kit (438888; Thermofisher) according to the manufacturer’s instructions. Power up™ SYBR® Green Master Mix (Applied Biosystem) was used to perform quantitative PCR. Primer sequences can be found in Supplementary Table 1. Results were calculated according to Pfaff et al. and are expressed as relative units compared to the control group. Quantitative PCR data were collected using QuantStudio Design & Analysis software v1.3.
and a High-Sensitivity Kit (Agilent Technologies) to ascertain the insert size, and the Qubit® (Life Technologies) was used to perform the fluorometric quantitation. Following validation, the libraries were normalised to 4 nM, pooled together and clustered on the cBot2 following the manufacturer’s recommendations. The pool was then sequenced using a 75-base paired-end (2 x 75 bp PE) dual index read format on the Illumina® HiSeq8000 according to the manufacturer’s instructions.

**RNA-seq analysis and differential gene expression.** Raw sequencing files were trimmed to remove adapter sequences and poor quality reads using TrimGalore. Trimmed reads were aligned with the STAR aligner (v2.5.1b) to the GRCh38 assembly of the human genome41. Raw counts were then calculated using Subread featureCounts (v1.5.1)43, and a differential expression analysis comparing glucose- and fructose-treated monocytes was performed using DESeq2 (Bioconductor)43. The resultant p value was corrected for multiple testing using the Benjamini and Hochberg method44. Differentially regulated genes were defined as those with a log₂, FC ≥ ±1 and an adjusted p value < 0.05. Heatmaps were generated using Morpheus (Broad Institute).

**Immunoblot**

**Human monocytes.** Monocyte lysate proteins were quantified, denatured and separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Polyvinylidene difluoride membranes were probed with antibodies targeting HKI (2024), HKII (2867), phospho-S6 ribosomal protein (pS6; 26235-236; 4858), phospho-AMPKα (2535), phospho-ACLY (4331), phospho-acetyl-CoA carboxylase 1 (3661), phospho-LDH (8176), pan-AKT (2920), phospho-AktSer473 (4060) and phospho-AktThr308 (4056). All antibodies were purchased from Cell Signaling (Danvers, MA) and used at a 1:1000 dilution. The total OXPHOS human cocktail (ab110411) and GLUT5 (ab41533) were purchased from Abcam and used at a 1:1000 dilution.

**Mouse BMDM.** Macrophages were lysed using RIPA buffer supplemented with 1% sodium dodecyl sulfate and phosphatase inhibitors (La Roche Ltd), denatured at 95 °C, and resolved on NuPAGE polyacrylamide pre-cast gels (Thermo Fisher Scientific) in the Supplementary information (Supplementary Fig. 9).

**Gas chromatography-mass spectrometry.** Isolated monocytes were incubated with heavily labelled 13C5-glutamine (2 mM; CLM-1822; Cambridge Isotopes) in RPMI media supplemented with 10% dialysed FBS (Thermo Fisher Scientific) before the transfer of gels onto nitrocellulose membranes using the iBlot2 (Invitrogen). Lysates were probed for phospho-S6 Ser235/236 (4858) ribosomal protein, total pS6 (clone S4D2; 2317), phospho-Akt Thr308 (clone 2449P; 4056), phospho-AktSer473 (clone D9E; 4060) and pan-Akt (clone 4D12; 2920). All antibodies were purchased from Cell Signaling Technologies. Secondary antibodies were purchased from LI-COR IRDye 800CW (donkey anti-mouse; 926-32212) and 680LT (donkey anti-rabbit; 926-68023). Fluorescence analysis was collected using the LI-COR Image Studio software v5.2. All original uncropped blots can be found in the Supplementary information (Supplementary Fig. 9).

**Liquid chromatography-mass spectrometry.** 13C5-glucose was purchased from Cambridge Isotope Laboratories and deuterated 13C6-fructose (2024), HKII (2867), phospho-S6 (pS6; 26235-236; 4858), phospho-AMPKα (2535), phospho-ACLY (4331), phospho-acetyl-CoA carboxylase 1 (3661), phospho-LDH (8176), pan-AKT (2920), phospho-AktSer473 (4060) and phospho-AktThr308 (4056). All antibodies were purchased from Cell Signaling (Danvers, MA) and used at a 1:1000 dilution. The total OXPHOS human cocktail (ab110411) and GLUT5 (ab41533) were purchased from Abcam and used at a 1:1000 dilution.

**Cellular metabolites were extracted and analysed by GC-MS using protocols described previously46,47. Metabolite extracts were derived using MTBSTFA (N-(tert-butyldimethylsilyl)-N-methyltrifluoracetamide), t-Myristic acid (750 ng/sample) was added as an internal standard to metabolite extracts, and metabolite abundance was expressed relative to the internal standard. GC/MS analysis was performed using an Agilent 5975C GC/MS equipped with a DB-5MS + DG (50 μm x 0.5 μm) capillary column (Agilent J&W, Santa Clara, CA, USA). Data were acquired using ChemStation E.02.02.1431. For SITA experiments, mass isotopomer distribution was determined using a custom algorithm developed at the McGill University46.

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Author contributions

N.J., J.B., F.Z., A.R., B.J.J. and A.I.M.B. performed experiments. N.J., J.B., F.Z., C.J.B., D. M., J.G.C., D.K.F., K.H.V., E.E.V. and C.A.T. designed the experiments and provided intellectual discussion. N.J., J.B., F.Z., D.G.H., C.J.B., D.A., G.W.I. and E.E.V. analysed the data. N.J., J.B., D.K.F., E.E.V. and C.A.T. wrote the manuscript. All authors critically revised and approved the manuscript.

Competing interests

K.H.V. is on the board of directors and shareholder of Bristol Myers Squibb, a shareholder of GRAIL, and on the science advisory board of PMV Pharma. R.A. is a consultant of Faeth Therapeutics, funded by Khosla Ventures. All other authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to E.E.V. or C.A.T.

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