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ELABORATO DI LAUREA

Il fruttosio assunto con la dieta induce lipogenesi epatica tramite produzione di acetato da parte del microbiota

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Sommario

ABSTRACT

Il consumo di fruttosio è aumentato notevolmente negli ultimi decenni a causa dell'utilizzo di saccarosio e sciroppo di mais ad alto contenuto di fruttosio nelle bevande e nei cibi processati, contribuendo all'aumento dei tassi di obesità e steatosi epatica non alcolica. L'assunzione di fruttosio stimola la lipogenesi *de novo* nel fegato, processo che consiste nella conversione di precursori carbonilici in acetil-CoA e poi in acidi grassi. Un ruolo fondamentale è svolto dall'enzima ATP citrato liasi (ACLY), che trasforma il citrato citosolico in acetil-CoA. Gli autori di questo articolo, utilizzando il tracciamento isotopico *in vivo*, mostrano che la delezione specifica di ACLY nel fegato dei topi non riesce a sopprimere la lipogenesi indotta dal fruttosio. Il fruttosio dietetico viene infatti convertito in acetato dal microbiota intestinale, e ciò fornisce acetil-CoA lipogenico indipendentemente dall'ACLY. La deplezione del microbiota o il silenziamento dell'enzima epatico ACSS2, che genera acetil-CoA dall'acetato, sopprime notevolmente la conversione del fruttosio in acetil-CoA e acidi grassi. I dati rivelano un meccanismo a doppia azione che regola la lipogenesi epatica, in cui la fruttolisi all'interno degli epatociti fornisce un segnale per promuovere l'espressione dei geni lipogenici e la generazione di acetato microbico alimenta i pool lipogenici di acetil-CoA.

STATO DELL'ARTE

Aumento del consumo di fruttosio e sindrome metabolica

La dieta e le abitudini correlate al cibo sono cambiate notevolmente negli ultimi decenni, sia nei Paesi industrializzati che in quelli in via di sviluppo. Lo stile di vita è mutato principalmente verso una diminuzione dell'attività fisica associata ad un introito eccessivo di calorie, ma anche in particolare verso un aumento della quantità di zuccheri aggiunti nelle bevande. I dolcificanti più comuni sono saccarosio (disaccaride di glucosio e fruttosio) e sciroppo di mais ad alto contenuto di fruttosio. Le bevande zuccherate ed energetiche costituiscono attualmente il 15-17% dell'apporto energetico giornaliero nella dieta Occidentale, superando del 5% il limite massimo di zuccheri semplici raccomandato dalle linee guida dell'Organizzazione Mondiale della Sanità. [1]

L'aumento del consumo di zuccheri (principalmente di fruttosio) a livello mondiale negli ultimi decenni è associato ad un parallelo incremento di obesità, insulino-resistenza (IR), diabete di tipo 2 (T2D) e steatosi epatica non alcolica (NAFLD), tutti fattori della cosiddetta Sindrome Metabolica. In particolare, analisi epidemiologiche condotte tra il 1990 e il 2019 in relazione all'incidenza mondiale di NAFLD mostrano che la prevalenza della malattia è aumentata del 50.4% in circa 3 decenni, dati in linea con la crescente epidemia globale di obesità e T2D. [2]

Il contributo del fruttosio alla patogenesi della NAFLD (che è la causa maggiore di malattie epatiche croniche nei Paesi Occidentali) è stato centro di ricerche intensive nell'ultimo decennio. La malattia si sviluppa quando l'apporto di lipidi al fegato eccede il tasso di smaltimento degli stessi (tramite β-ossidazione e secrezione di trigliceridi sotto forma di particelle lipoproteiche). Prove sempre più numerose indicano come l'aumento della lipogenesi *de novo* (DNL) epatica contribuisca in maniera significativa allo sviluppo della NAFLD. La DNL è un processo metabolico mediante il quale il corpo sintetizza nuovi acidi grassi (lipidi) da fonti non lipidiche, come carboidrati e proteine. Questo processo avviene principalmente nel fegato e nei tessuti adiposi quando vi è un eccesso di energia sotto forma di glucosio o altri substrati. La DNL è una via altamente regolata, in cui i livelli di attività degli enzimi chiave possono aumentare in risposta al fruttosio alimentare. Tale zucchero stimola infatti l'attività di SREBP1c (*sterol regulatory element-binding transcription factor 1c*) e ChREBP (*carbohydrate response element-binding protein*), importanti attivatori trascrizionali della DNL, indipendentemente dalla segnalazione insulinica. In particolare, ChREBP attiva nel fegato geni glicolitici e lipogenici, agendo a feedback per incrementare ulteriormente la sua stessa l'attività, nonché la DNL e la secrezione di VLDL (lipoproteine a densità molto bassa). [1]

Il metabolismo del fruttosio

Per capire come il fruttosio possa influire sulla lipogenesi e quindi indurre steatosi epatica, bisogna analizzare i prodotti del suo metabolismo e i destini a cui essi possono andare incontro. Il primo step del metabolismo intracellulare del fruttosio è mediato dall'enzima chetoesochinasi (KHK), conosciuto anche come fruttochinasi, che converte il fruttosio in fruttosio 1-fosfato. Il secondo enzima che interviene nella fruttolisi è la fruttosio-bifosfato aldolasi (*Aldo*), che esiste nelle isoforme A, B e C (espresse in tessuti diversi) e scinde il fruttosio 1-fosfato in diidrossiacetone fosfato (DHAP) e gliceraldeide (GA). A questo punto, la triochinasi/FMN ciclasi (TKFC) può trasformare la GA in gliceraldeide 3-fosfato (GA3P), la quale può essere a sua volta catabolizzata dall'aldeide deidrogenasi (ALDH) per formare piruvato, che è anche il prodotto finale della glicolisi. Un terzo destino metabolico a cui può andare incontro la GA derivata dal fruttosio è la sua conversione in glicerolo da parte dell'alcol deidrogenasi (ADH); il glicerolo viene poi fosforilato dalla glicerolo chinasi (GK) per formare glicerolo 3-fosfato. [3]

Le vie metaboliche di fruttosio e glucosio convergono quindi verso intermedi comuni, che possono essere utilizzati per assemblare lo scheletro degli acidi grassi. Nonostante le varie interconversioni e convergenze di glicolisi e fruttolisi, più atomi di carbonio derivati dal fruttosio vengono incorporati in lattato e trigliceridi rispetto al glucosio, suggerendo come il flusso di carbonio attraverso le due vie metaboliche avvenga in modo diverso. Infatti, la glicolisi è regolata dall'insulina, dal citrato e dall'ATP, che influenzano l'attività della fosfofruttochinasi (enzima che catalizza lo step glicolitico di conversione del fruttosio 6-fosfato in fruttosio 1,6-bifosfato), mentre la fruttolisi non è modulata né dall'insulina né dai prodotti finali del metabolismo dei carboidrati. Inoltre, nel fegato la fosforilazione del fruttosio da parte di KHK è 10 volte più veloce della fosforilazione del glucosio da parte della glucochinasi (GCK). Gli enzimi che identificano in maniera specifica le prime reazioni della fruttolisi, assieme al rapido flusso non regolato di carbonio attraverso tale via metabolica, potrebbero dare ragione degli effetti nettamente differenti che fruttosio e glucosio hanno sul metabolismo dell'intero organismo. [3]

Il fruttosio è assorbito attraverso l'epitelio intestinale per trasporto facilitato, principalmente tramite i trasportatori GLUT2 e 5, espressi sulla superficie apicale degli enterociti (di cui GLUT5 è il principale ed è altamente specifico per il fruttosio). GLUT2 è localizzato anche a livello della membrana baso-laterale ed è responsabile del trasferimento del fruttosio dal citoplasma dell'enterocita al sangue. Il metabolismo intestinale del fruttosio è regolato da un meccanismo feed-forward: topi sottoposti a somministrazione forzata di fruttosio esibiscono un'espressione aumentata dei geni fruttolitici, come GLUT5, KHK e *Aldo*B; quest'effetto è mediato dai prodotti del metabolismo del fruttosio, come dimostra il fatto che il knockout di KHK previene la sovra-regolazione di GLUT5. A livello trascrizionale, gli intermedi fruttolitici vanno ad alimentare l'attività di ChREBP, che regola, tra l'altro, l'espressione di geni coinvolti nel trasporto e nel metabolismo del fruttosio. [3]

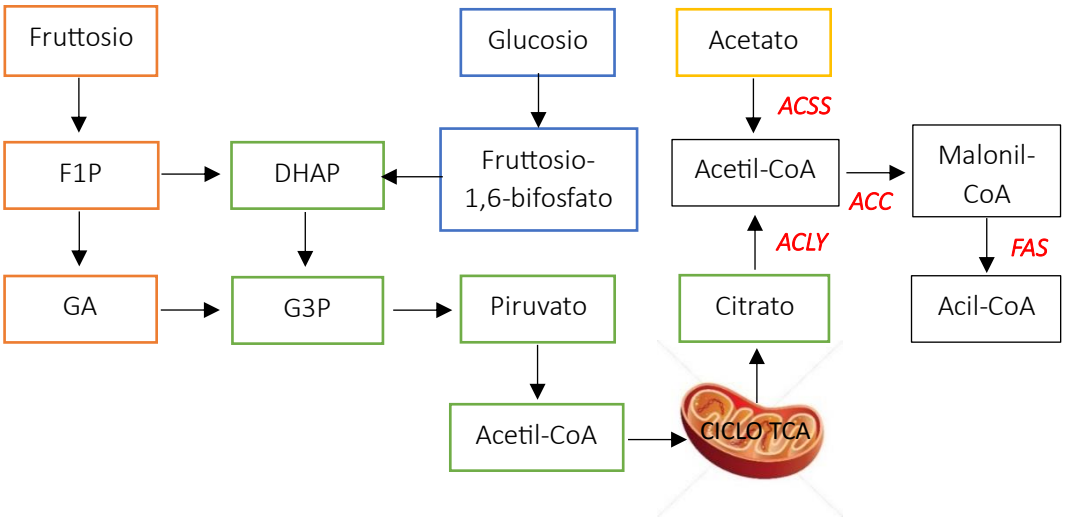
Tradizionalmente, il fegato è sempre stato considerato il principale organo atto al metabolismo del fruttosio. Recentemente, usando traccianti isotopici stabili, è stato dimostrato che l'intestino tenue, soprattutto il digiuno, presenta il più alto livello di fruttosio 1-fosfato, metabolizzando circa il 90% del fruttosio introdotto tramite la dieta (3-5 g). La piccola parte di fruttosio alimentare che arriva al fegato tramite la circolazione portale serve soprattutto per accendere il macchinario della lipogenesi (ad esempio tramite induzione di SREBP1c). Lo zucchero entra negli epatociti non attraverso GLUT5 ma principalmente tramite GLUT2 e GLUT8, e una volta nel citoplasma viene rapidamente fosforilato da KHK-C; l'espressione di questo enzima è sovra-regolata dal fruttosio stesso tramite ChREBP, che nel fegato regola anche l'espressione di geni coinvolti nella DNL. [3]

La lipogenesi *de novo*

Per comprendere come il fruttosio possa indurre nel fegato la lipogenesi *de novo*, analizziamo brevemente il processo di sintesi degli acidi grassi. La DNL può essere suddivisa in 3 passaggi sequenziali: sintesi degli acidi grassi (FA), elongazione delle catene di FA, e assemblaggio a formare trigliceridi. Sia la glicolisi che la fruttolisi convergono nella produzione di intermedi tricarbonilici, ovvero GA3P e DHAP, i quali possono essere ulteriormente metabolizzati a formare piruvato. Il piruvato può quindi entrare nel mitocondrio, dove viene convertito in acetil-CoA per essere utilizzato nel ciclo degli acidi tricarbossilici (TCA) e generare ATP. Quando non c'è necessità di produrre altra energia, gli intermedi TCA si accumulano, e il citrato (uno degli intermedi del ciclo) è condotto dal mitocondrio al citoplasma tramite specifici meccanismi di trasporto. Tale intermedio viene quindi convertito in acetil-CoA dall'enzima ACL (o ACLY) (*adenosina triphosphate citrate lyase*). Il citrato funge anche da attivatore allosterico di ACC (*cytoplasmic acetyl-CoA carboxylase*), enzima che converte l'acetil-CoA in malonil-CoA, iniziando così la DNL. Il malonil-CoA sarà utilizzato dal complesso FAS (*fatty acid synthase*) per estendere di due carboni la catena acilica in elongazione, formando alla fine palmitato, un acido grasso saturo di 16 atomi di carbonio; tale prodotto primario può essere allungato fino a 18C per generare l'acido stearico. Entrambi questi acidi possono poi essere desaturati e/o esterificati ad uno scheletro di glicerolo fosfato per formare lipidi più complessi come i trigliceridi. [4]

La regolazione trascrizionale della neo-sintesi degli acidi grassi avviene attraverso due principali vie attivanti, modulate da SREBP1c e ChREBP, fattori di trascrizione stimolati rispettivamente da un aumento del segnale insulinico e da un aumento della concentrazione di glucosio, entrambi indotti dall'assunzione di cibo. Come evidenziato precedentemente, anche il fruttosio può tuttavia attivare queste vie regolative. Inoltre, nonostante il glucosio sia riconosciuto come il substrato standard della DNL, anche il fruttosio risulta essere un substrato altamente lipogenico. La G3P e il DHAP prodotti dal metabolismo del fruttosio possono entrare in glicolisi, consentendo la produzione di acetil-CoA e promuovendo la lipogenesi in modo indipendente dalla regolazione insulinica. [5]

È importante evidenziare poi che l'acetil-CoA può essere generato anche a partire dall'acetato tramite l'acetil-CoA sintetasi (ACSS), sia nel mitocondrio (ACSS1) che nel citosol (ACSS2). La fonte primaria di acetato esogeno è il microbiota intestinale: i carboidrati non digeriti vengono metabolizzati tramite processi fermentativi dai batteri enterici, per generare acetato, propionato e butirrato in rapporto stechiometrico 3:1:1. Questi acidi grassi a catena corta sono assorbiti nel colon e sono responsabili approssimativamente del 10% della spesa energetica totale dell'uomo. L'acetato può in realtà derivare anche direttamente da fonti alimentari, oppure dal metabolismo epatico dell'etanolo (che ne costituisce una fonte considerevole), nonché da fonti endogene come la deacetilazione delle proteine istoniche. [6]



Dunque, come si può vedere dallo schema soprastante, l'enzima ACLY svolge un ruolo chiave nella produzione di acetil-CoA a partire dai prodotti del metabolismo cellulare del fruttosio nel fegato. Tale acetil-CoA può essere utilizzato per la sintesi di acidi grassi, portando all'accumulo di lipidi nel tessuto epatico. L'eliminazione genetica di ACLY negli epatociti potrebbe pertanto influenzare questo processo impedendo la produzione di acetil-CoA e di conseguenza riducendo la DNL epatica. Nell'articolo che si andrà ad analizzare, S. Zhao e coautori hanno ipotizzato che inducendo nei topi il knockout del gene ACLY, si potrebbe evitare l'accumulo eccessivo di lipidi nel fegato causato dall'assunzione di fruttosio. Quello che i ricercatori hanno scoperto è che, in una dieta ricca di fruttosio, il maggior contributo alla lipogenesi epatica è dato in realtà dall'acetato derivato dal metabolismo batterico di tale zucchero. Questo substrato fornisce i carboni per le catene aciliche, mentre i prodotti del metabolismo epatico del fruttosio assorbito nell'intestino tenue promuovono l'espressione degli enzimi coinvolti nella DNL.

ANALISI DEI METODI

Western blotting

Il Western blot è un metodo spesso utilizzato in ricerca per separare e identificare le proteine. In questa tecnica, una miscela di peptidi viene divisa in base al peso molecolare, e quindi per tipo, mediante elettroforesi su gel. Le proteine, raggruppate in bande, vengono poi trasferite dal gel su una membrana, la quale viene successivamente incubata con anticorpi specifici per la proteina d'interesse; questi vengono poi rilevati, consentendo di visualizzare la banda corrispondente al peso molecolare del peptide target.

Le cellule devono essere innanzi tutto lisate. A partire dal surnatante del buffer di lisi, si può poi misurare la concentrazione proteica usando uno spettrofotometro. Per ogni campione da analizzare, a partire dal corrispettivo rapporto massa/volume di proteine, si calcola la quantità di soluzione da porre nel pozzetto di elettroforesi per far sì che in ciascuno di essi ci sia la stessa quantità totale di proteine. Si prepara poi la vaschetta e il gel per l'elettroforesi, nei cui pozzetti vengono caricati i vari campioni e il marcatore di peso molecolare. [7]

Dopo aver separato la miscela di proteine in corsa elettroforetica, essa viene trasferita su una membrana utilizzando un campo elettrico orientato perpendicolarmente alla superficie del gel. La membrana (che può essere o nitrocellulosa o PVDF) viene posizionata tra la superficie del gel e l'elettrodo positivo, in mezzo fra due strati di spugna e di carta da filtro che proteggono il gel (vedi *figura 1*). Dopo il trasferimento, la membrana va incubata per una notte in un agitatore con una soluzione di anticorpi primari in siero bovino di albumina (BSA). Prima dev'essere però effettuato il "blocking", procedimento che impedisce agli anticorpi di legarsi alla membrana in maniera aspecifica. Gli anticorpi possono essere visualizzati facendo uso di enzimi come la perossidasi di rafano, che danno un segnale in corrispondenza della posizione della proteina target; tale segnale viene impresso su una pellicola, poi sviluppata in una camera oscura. [7]

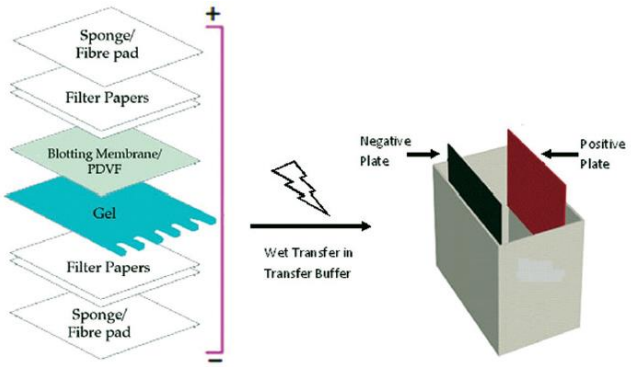


Figura 1

RT-qPCR

La PCR quantitativa (qPCR, detta anche Real-Time PCR) a trascrizione inversa (RT) consente di quantificare l'abbondanza di un acido nucleico monitorando la fluorescenza emessa dalla reazione di amplificazione di una certa quantità di templato iniziale. Questa tecnica è stata applicata in molti campi di ricerca per analizzare le differenze nell'abbondanza di un mRNA target tra campioni provenienti da diversi gruppi/trattamenti. La quantificazione relativa dell'mRNA richiede la selezione di geni normalizzatori (di riferimento o *housekeeping*), espressi in modo stabile tra repliche biologiche e gruppi/trattamenti differenti. Viene definita qPCR run la procedura che consente di ottenere il ciclo soglia (Ct/Cq) per un gene di interesse (GOI) o un gene normalizzatore, in tutte le repliche biologiche; il Ct è il ciclo di PCR in corrispondenza del quale la fluorescenza data dal SYBR Green legato al DNA (la quale è proporzionale alla quantità di acido nucleico in soluzione) aumenta fino a sovrastare il rumore di fondo ed essere quindi rilevabile. [8]

La qPCR si basa sulla correlazione che sussiste tra il numero di cicli di amplificazione necessari affinché il prodotto di PCR diventi rilevabile (Ct) e la quantità di templato presente all'inizio nel campione: maggiore è la concentrazione iniziale di DNA, minore è il numero di cicli necessario a rilevare la fluorescenza (se in un campione è presente un numero di molecole doppio rispetto ad un altro campione, esso giungerà alla soglia un ciclo in anticipo rispetto al secondo).

Il DNA che sarà amplificato in qPCR deriva dall'mRNA totale estratto dal campione, che viene retro-trascritto per sintetizzare cDNA. Il mix di priming per la reazione di trascrizione inversa (RT) comprende random primers o oligo-(dT) e dNTPs (deossinucleotidi). In ogni tubino di PCR si pone l'RNA proveniente da un replicato biologico corrispondente ad una certa condizione addizionato al mix di priming RT; il tutto va incubato in un termociclatore per consentire ai primers di appaiarsi al templato. Il mix di reazione RT comprende invece: DTT (composto che protegge l'attività enzimatica della trascrittasi inversa), RT buffer e trascrittasi inversa in soluzione; questo mix va aggiunto ai tubini di PCR dopo la fase di priming, e va incubato alla temperatura ottimale per la reazione di trascrizione inversa. Successivamente, si procede con la qPCR, il cui mix di reazione è costituito dalla soluzione di cDNA risultante dalla RT, i primers di PCR, e il mix di SYBR Green (intercalante per la rilevazione di molecole di dsDNA). Il programma di real-time PCR prevede una fase di iniziazione e poi 40 cicli di amplificazione, alla fine di ognuno dei quali si misura la fluorescenza: il ciclo a cui questa diventa rilevabile corrisponde al ciclo soglia. [8]

Quando si confronta l'espressione di un certo gene target in tessuti/condizioni differenti, è importante normalizzare i risultati ottenuti facendo riferimento a un gene *housekeeping*. Per ciascuna condizione va perciò calcolata la differenza tra il ciclo soglia relativo al gene target (*sample*) e quello relativo al gene normalizzatore (*reference*): $\Delta C_{T\ GOI} = C_{T\ reference} - C_{T\ sample}$ [8]

Tracciamento con isotopi stabili

Si definiscono isotopi di un certo elemento chimico le varianti di tale elemento che presentano lo stesso numero atomico ma diverso numero di massa, ovvero diverso numero di neutroni nel nucleo. Storicamente, gli esperimenti di tracciamento per la misurazione della sintesi lipidica utilizzavano radioisotopi come acqua marcata con trizio (^{3}H) e acetato o acidi grassi marcati con carbonio-14 (^{14}C). ^{3}H e ^{14}C sono isotopi radioattivi, ovvero atomi instabili che subiscono decadimento radioattivo nel tempo: il nucleo emette particelle (alfa o beta) oppure energia sotto forma di radiazione gamma, finché l'isotopo originale si trasforma in un nuovo elemento o isotopo stabile. Le informazioni metaboliche ottenute dalla ricerca con traccianti a radioisotopi hanno gettato le basi per la ricerca con traccianti a isotopi stabili (come ^{13}C e ^{2}H), ovvero varianti di un elemento chimico che non subiscono decadimento radioattivo nel tempo, e quindi consentono di condurre studi di routine in modo molto più semplice e senza precauzioni speciali. [9]

L'uso di traccianti basati sul carbonio (come acetato marcato con ^{13}C) consente di arricchire il pool di acetil-CoA con precursori "etichettati", i quali passeranno tale etichetta (ovvero i carboni isotopici) ai vari intermedi della catena di reazione della DNL. Tramite un approccio noto come analisi della distribuzione degli isotopomeri di massa (MIDA), è possibile determinare il tasso di sintesi di un polimero (come può essere considerato l'acido grasso) basandosi sul rapporto di arricchimento dello stesso, ovvero il numero di ^{13}C contenuti nella molecola rispetto al totale. L'arricchimento isotopico di un composto può essere determinato tramite metodi per la misurazione del peso molecolare, come la spettrometria di massa (tecnica descritta in seguito). Il peso molecolare dipende infatti dalla massa atomica degli elementi che costituiscono il composto, la quale varia tra isotopi diversi di uno stesso elemento. [9]

L'acqua deuterata ($^{2}H_2O$) è un tracciante alternativo per la lipogenesi, che sta trovando sempre più impiego grazie ai diversi vantaggi che presenta rispetto ai traccianti a base di carbonio. In questo caso sono gli idrogeni dell'acqua ad essere marcati (deuterio): nel contesto della DNL, essi saranno in parte direttamente incorporati nell'acetil-CoA e in parte utilizzati nei successivi passaggi di idratazione e riduzione operati da FAS, enzima che catalizza la polimerizzazione degli acil-CoA per formare acidi grassi a catena lunga. Oltre ad essere relativamente poco costosa, l'acqua deuterata si equilibra rapidamente con l'intero volume di acqua corporea, può essere somministrata facilmente per lunghi periodi (necessari per lo studio della cinetica dei trigliceridi adiposi e degli acidi grassi), non richiede infusioni endovenose, e permette la misurazione simultanea di diverse reazioni metaboliche (tra cui la sintesi delle catene aciliche dei trigliceridi). Questo tipo di tracciamento non consente però di distinguere, nel pool lipidico totale, gli acidi grassi derivanti da uno specifico substrato iniziale. [9]

LC-MS

La LC-MS è una tecnica che combina la cromatografia liquida (LC) con la spettrometria di massa (MS), di cui la prima consiste nella separazione fisica dei composti contenuti in una soluzione campione, mentre la seconda consente di suddividere questi composti secondo la loro massa.

Nella cromatografia liquida, pochi microlitri di soluzione vengono iniettati all'interno di un flusso continuo di solvente, chiamato fase mobile. La fase mobile viene pompata attraverso una colonna (un tubo di acciaio inossidabile) riempita con particelle di silice rivestite da un altro liquido, chiamato fase stazionaria. Quando la soluzione del campione miscelata alla fase mobile raggiunge la colonna, i suoi componenti (analiti) interagiscono in modo differenziale con la fase stazionaria a seconda della loro composizione chimica o delle loro proprietà fisiche. Gli analiti che si legano più debolmente emergono per primi dalla colonna, mentre quelli rimanenti affiorano in seguito e in maniera sequenziale (fintanto che la fase mobile continua a fluire attraverso la colonna); per ultimi emergono i composti che stabiliscono le interazioni più forti con la fase stazionaria. Il tempo che uno specifico analita trascorre nella colonna è caratteristico di quell'analita ed è chiamato tempo di ritenzione (RT). La soluzione che fuoriesce dalla colonna passa attraverso un rivelatore che "risponde" a una determinata proprietà fisica o chimica (come l'indice di rifrazione o l'assorbimento della luce) degli analiti in essa contenuti. Questa risposta fornisce un segnale (interpretato come un picco) la cui intensità (area o altezza del picco) corrisponde alla quantità del composto nel campione. Il momento in cui il rivelatore rileva l'analita è il suo tempo di ritenzione (RT): l'identità di un certo analita può essere confermata confrontando il suo RT con quello di un composto noto. [10]

Tra i vari rivelatori che possono essere accoppiati con la cromatografia liquida, lo spettrometro di massa (*figura 2*) si è affermato essere particolarmente selettivo, sensibile e universale. Mentre il sistema LC funziona a pressioni ambientali, la MS funziona sottovuoto; perciò, i due sono collegati tramite un'interfaccia. Man mano che l'eluente della colonna fluisce nell'interfaccia, il solvente viene fatto evaporare fornendo calore, mentre le molecole degli analiti vengono vaporizzate e ionizzate: lo spettrometro di massa è infatti in grado di rilevare e misurare solo ioni in fase gassosa. Gli ioni degli analiti vengono successivamente aspirati nello spettrometro, dove sono sottoposti a campi elettrici e magnetici che fanno curvare le particelle cariche in modo dipendente dal loro rapporto massa/carica (m/z). Dopo la separazione, gli ioni possono essere raccolti e rilevati da una varietà di rivelatori di massa, di cui il più comune è il moltiplicatore di elettroni; si tratta un dinodo (superficie metallica) che quando viene colpito dagli ioni separati dalla MS rilascia elettroni secondari, i quali vengono moltiplicati facendoli passare attraverso una serie di altri dinodi. La corrente amplificata generata dal flusso di elettroni secondari viene misurata e correlata alle concentrazioni dei vari ioni nello spettrometro di massa. [10]

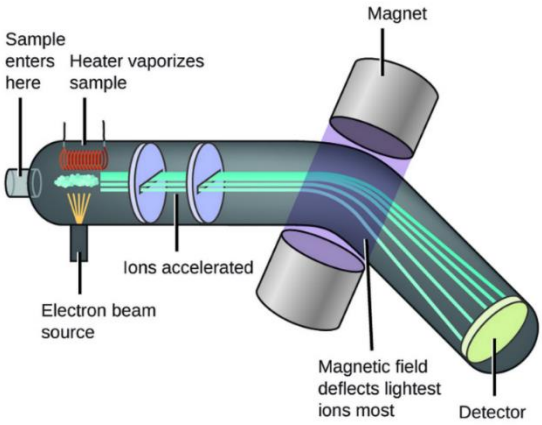


Figura 2

("Atomic Structure and Symbolism: Figure 5" by OpenStax Chemistry, CC BY 4.0)

La spettrometria di massa può essere utilizzata per determinare la composizione isotopica delle molecole; questo perché gli analiti ionizzati vengono separati secondo il loro rapporto massa/carica: dato che isotopi diversi dello stesso elemento presentano masse atomiche differenti, composti uguali formati da isotopi diversi devieranno in maniera differente all'interno dei campi elettro-magnetici applicati.

Tecniche istologiche

I campioni di tessuto possono essere osservati utilizzando diverse tecniche di colorazione, al fine di evidenziare le strutture più rilevanti per lo specifico studio che si sta svolgendo. Le due tecniche istologiche utilizzate negli esperimenti descritti nell'articolo in analisi sono le seguenti.

- Ematossilina-eosina (H&E): la soluzione di ematossilina va ad evidenziare la cromatina nucleare, e possibilmente altri elementi cellulari acidi; l'eosina colora invece di rosa il citoplasma della cellula e la matrice extracellulare.
- Olio rosso O (Oil Red O): è un colorante liposolubile e idrofobico che colora di rosso i grassi neutri, gli acidi grassi e i trigliceridi, ma non è in grado di rilevare in maniera efficace fosfolipidi e glicolipidi, i quali presentano gruppi polari (perciò non colora le membrane biologiche).

DISCUSSIONE

Come menzionato nell'introduzione, l'enzima ACLY contribuisce alla lipogenesi *de novo* (DNL) nel fegato, catalizzando la reazione di conversione del citrato in acetil-CoA. Il citrato è un intermedio del ciclo degli acidi tricarbossilici, il quale è alimentato anche dagli intermedi prodotti dal metabolismo del fruttosio (GA3P e DHAP, convertiti in piruvato). Gli autori dell'articolo hanno osservato che, sebbene l'eliminazione completa di ACLY sia letale nell'embrione, i topi in cui è stato fatto knockout dell'enzima esclusivamente nel tessuto epatico (denominati *Liver Acly KnockOut* - LAKO) non presentano differenze evidenti rispetto ai topi di controllo wild-type (WT) per quanto riguarda il peso corporeo e le dimensioni degli organi, sia con una dieta a base di cibo standard (CD- *chow diet*) che con una dieta ad alto contenuto di fruttosio (60%) (HFrD). Nelle immagini istologiche in *figura 3* si nota un aumento del contenuto lipidico degli epatociti (bianco) dopo qualche settimana di HFrD, in misura del tutto comparabile tra topi LAKO e WT; le differenze legate al genotipo sono minime. Tali dati dimostrano che, inaspettatamente, l'assenza di ACLY non influisce significativamente sui livelli globali di metaboliti epatici né impedisce l'accumulo di trigliceridi causato dall'assunzione di fruttosio.

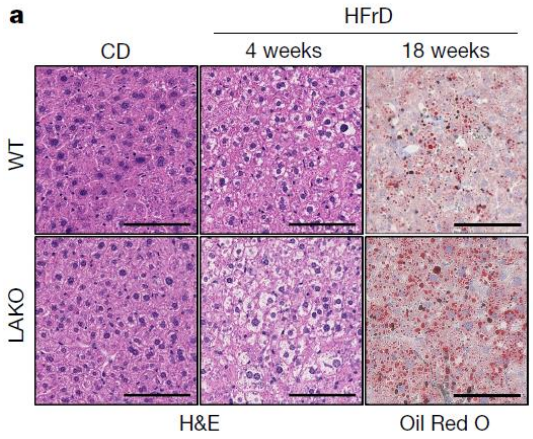


Figura 3. Colorazione istologica di fegato con Ematossilina-Eosina e Olio Rosso O, da topo WT e topo LAKO nutriti con CD o HFrD per 4 o 18 settimane.

Risultati del tutto analoghi sono stati ottenuti nutrendo i topi con una dieta standard ma somministrando loro acqua potabile contenente una miscela 1:1 di fruttosio e glucosio (15% ciascuno): gli animali che bevevano tale soluzione per 4 settimane sviluppavano una lieve steatosi epatica.

Date queste scoperte inaspettate, si è voluto testare direttamente l'effetto della carenza di ACLY sulla conversione del fruttosio in acidi grassi. La soluzione fruttosio:glucosio è stata marcata in uno dei due zuccheri con carbonio-13 (^{13}C), ed è stata somministrata sia ai topi wild-type e che ai topi LAKO. Dalle analisi condotte successivamente (*figura 4*) è risultato che i carboni del fruttosio erano stati incorporati negli acidi grassi di entrambe le classi genotipiche in misura simile. I

carboni del glucosio hanno contribuito molto meno alla costituzione delle catene aciliche rispetto a quelli del fruttosio, senza notevoli differenze tra genotipi.

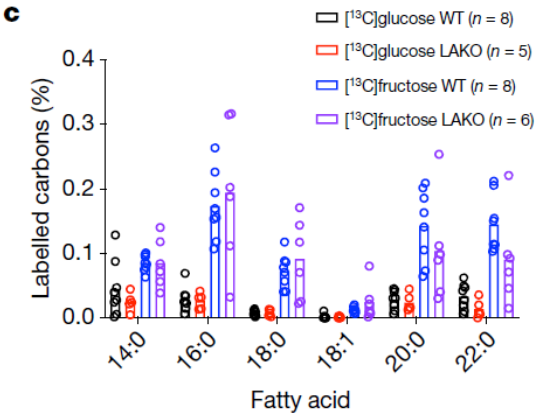


Figura 4. Percentuali di carbonio totale etichettato (marcato con ^{13}C) in acidi grassi saponificati prelevati dal siero di topi nutriti con fruttosio:glucosio, di cui uno dei due zuccheri marcato con ^{13}C.

Questi dati indicano che, contrariamente ai modelli esistenti del metabolismo del fruttosio, l'uso dei carboni di questo zucchero per la DNL epatica non richiede l'enzima ACLY. Non è quindi possibile che tale processo dipenda esclusivamente dalla conversione dei prodotti della fruttolisi (GA3P e DHAP) in piruvato, e dalla successiva conversione di quest'ultimo in citrato e poi in acetil-CoA tramite ACLY.

Il fatto che la DNL si sia dimostrata indipendente da ACLY ha spostato l'attenzione dei ricercatori su ACSS2, enzima citosolico che converte l'acetato in acetil-CoA. Nel contesto di una dieta ricca di fruttosio, l'espressione di ACSS2 risulta notevolmente aumentata nei topi LAKO rispetto ai controlli WT, come constatabile in *figura 5*.

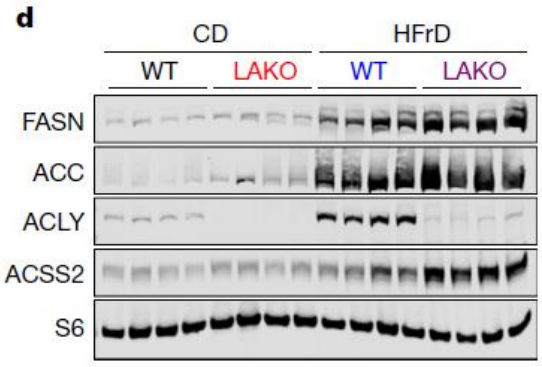


Figura 5. Western blot di enzimi lipogenici prelevati tramite lisi del tessuto epatico di topi WT e LAKO nutriti con CD o HFrD per 4 settimane; la proteina ribosomiale S6 è stata usata come controllo di caricamento.

Questi dati suggeriscono che la conversione dell'acetato in acetil-CoA da parte di ACSS2 possa supportare la DNL in assenza di ACLY, facendo quindi dell'acetato una fonte importante di substrati per la lipogenesi nel contesto del consumo di fruttosio. Dato che questo intermedio può essere generato all'interno delle cellule dei mammiferi attraverso vari meccanismi, Zhao e coautori hanno indagato la possibilità che il fruttosio venga convertito in acetato in modo autonomo negli epatociti. L'incubazione di cellule isolate dal fegato di topo WT in una soluzione al 25mM di fruttosio [^{13}C] ha portato alla formazione di intermedi fruttolitici marcati (in rosso in *figura 6b*), ma solo di una minima quantità di acetil-CoA e malonil-CoA (in rosso in *figura 6c*), a dimostrazione del fatto che i principali substrati della DNL non derivino dal fruttosio metabolizzato nell'epatocita. Al contrario, incubando le cellule WT in una soluzione 1mM di acetato [^{13}C], si osserva la formazione di acetil-CoA e malonil-CoA marcati (in blu in *figura 6c*). Tali risultati provano che i pool lipogenici di acetil-CoA negli epatociti derivano per la maggior parte da acetato esogeno, elaborato in una via metabolica indipendente da ACLY.

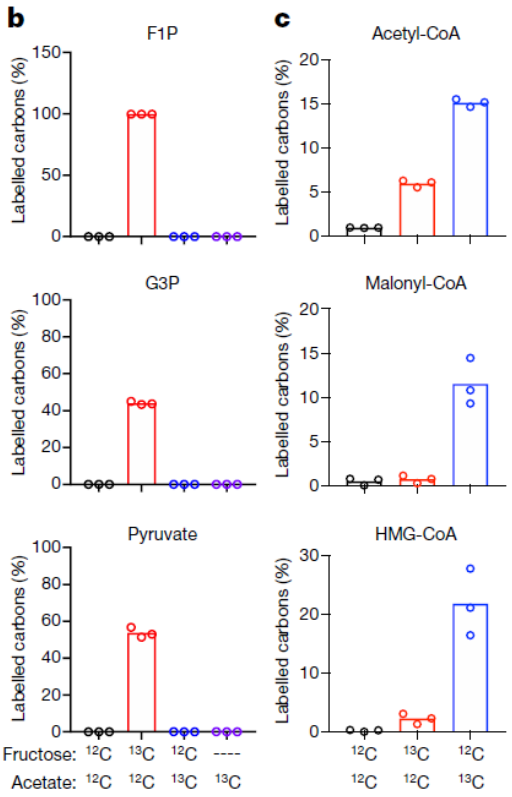


Figura 6. Percentuali di carbonio totale etichettato in intermedi fruttolitici (pannello b) e substrati lipogenetici (c) in cellule epatiche incubate con fruttosio/acetato [^{13}C].

Si è quindi esplorata l'ipotesi che il fruttosio venga convertito in acetato prima di raggiungere il fegato per alimentare la DNL epatica. Questo è stato fatto tramite un'analisi di tracciamento isotopico del fruttosio [^{13}C] nel tempo, riportata in *figura 7*. L'amministrazione orale nel topo di fruttosio [^{13}C] ha portato alla rilevazione sia di fruttosio 1-fosfato (F1P) che di piruvato marcati nel fegato 15-30 minuti dopo l'introduzione, ad indicare rapido assorbimento e fruttolisi epatica. L'acetil-CoA epatico ha invece raggiunto il picco molto più lentamente, dopo 60-90 minuti dall'introduzione del fruttosio, e questa cinetica lenta è risultata strettamente correlata all'apparizione di acetato etichettato nella circolazione portale (che trasferisce le sostanze assorbite nell'intestino al fegato). L'etichettatura degli acidi grassi epatici ha seguito quella dell'acetil-CoA, raggiungendo il picco a 120-180 minuti. Questi risultati mostrano come l'alimentazione con fruttosio comporti la sintesi di acetil-CoA e acidi grassi epatici in maniera indiretta, ovvero non a partire dai metaboliti derivanti dalla fruttolisi epatica, bensì dall'acetato prodotto dal fruttosio in maniera esogena (nell'intestino) e poi trasportato al fegato.

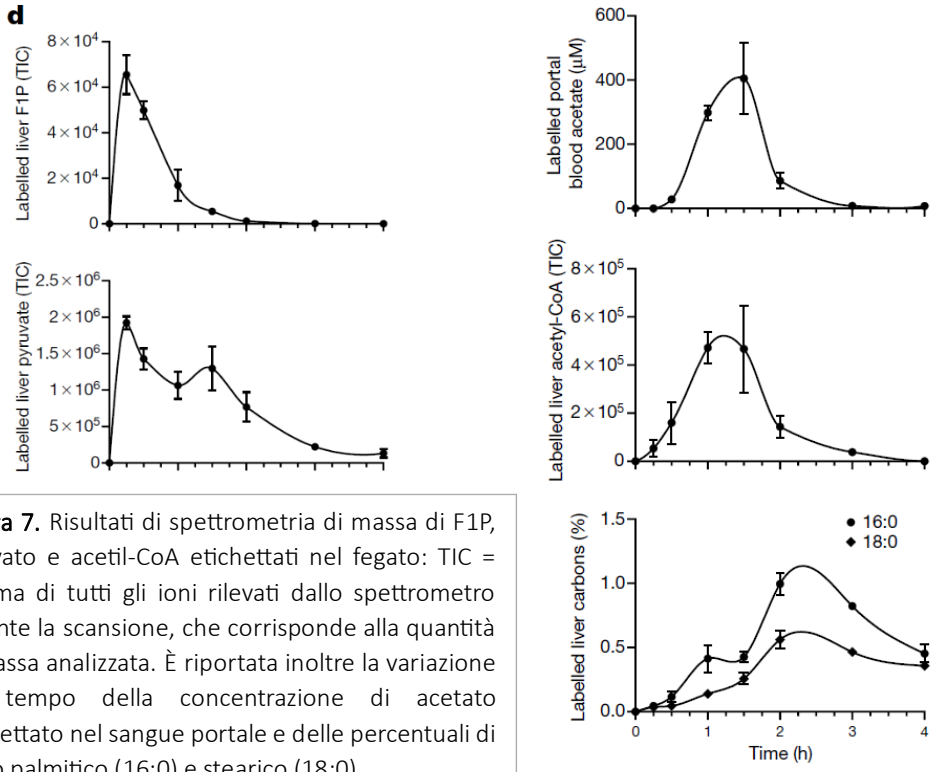


Figura 7. Risultati di spettrometria di massa di F1P, piruvato e acetil-CoA etichettati nel fegato: TIC = somma di tutti gli ioni rilevati dallo spettrometro durante la scansione, che corrisponde alla quantità di massa analizzata. È riportata inoltre la variazione nel tempo della concentrazione di acetato etichettato nel sangue portale e delle percentuali di acido palmitico (16:0) e stearico (18:0).

Zhao e coautori hanno dunque cercato di determinare quale sia la fonte dell'acetato derivato dal fruttosio. Sebbene questo zucchero venga principalmente assorbito nell'intestino tenue, il fruttosio non assimilato raggiunge il colon, dove i batteri del microbiota lo convertono in acidi grassi a catena corta, tra cui l'acetato. Per testare se il microbiota svolga un ruolo importante per la DNL epatica, i topi sono stati trattati con un cocktail di antibiotici, così da eliminare tutti i batteri. In questi animali, a seguito di somministrazione orale di fruttosio [^{13}C], l'etichettatura di F1P, piruvato e citrato a livello epatico è rimasta invariata, mentre quella dell'acetil-CoA e del palmitato si è notevolmente ridotta. D'altro canto, l'espressione dei geni della DNL è rimasta intatta dopo il trattamento con antibiotici; questo perché l'induzione di tali geni dopo il consumo di fruttosio dipende da intermedi fruttolitici e/o glicolitici derivanti dal metabolismo epatico, che, in linea con il normale passaggio del fruttosio dall'intestino al fegato, si è mantenuto invariato.

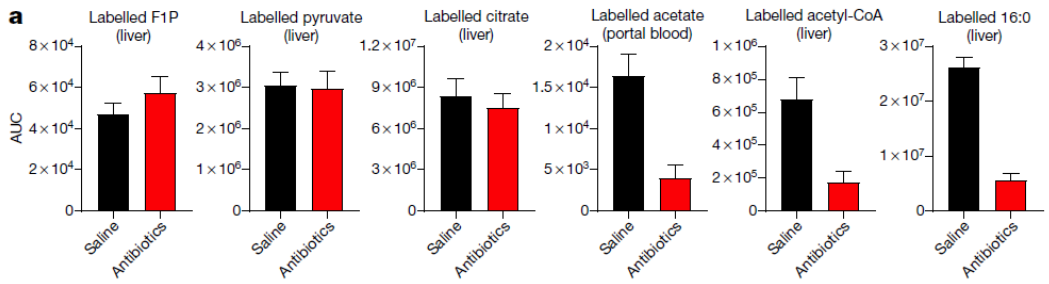


Figura 8. AUC = *area under curve*, relativa alla misurazione in spettrometria di massa dei metaboliti sopra indicati; equivale alla quantità di composti marcati dalla presenza di ^{13}C in topi wild-type trattati con soluzione salina o con antibiotici e nutriti con una soluzione 1:1 di fruttosio [^{13}C] e glucosio non marcato.

Per verificare che a supportare la DNL fosse effettivamente l'acetato prodotto dal metabolismo microbico, ai topi è stato somministrato acetato [^{13}C] in associazione a fruttosio:glucosio. La DNL da acetato [^{13}C], diversamente da quella da fruttosio [^{13}C], ha dimostrato non essere influenzata dal trattamento con antibiotici. Al fine di confermare che l'ACSS2 epatico, che converte l'acetato in acetil-CoA, è essenziale per il sostentamento della lipogenesi *de novo* (DNL) innescata dal fruttosio, è stato soppresso l'enzima nel fegato mediante l'utilizzo di un vettore AAV (*adeno-associated virus*) contenente un hairpin, ovvero una molecola di RNA a forcina che va a silenziare il gene target tramite RNA *interference*. Il knock-down dell'ACSS2 epatico ha soppresso fortemente l'etichettatura degli acidi grassi derivanti da fruttosio [^{13}C]. Questi dati, riportati nei grafici sottostanti, confermano che il substrato lipogenico principale nel fegato è l'acetato prodotto dal fruttosio tramite il microbiota, convertito in acetil-CoA dall'ACSS2 epatico.

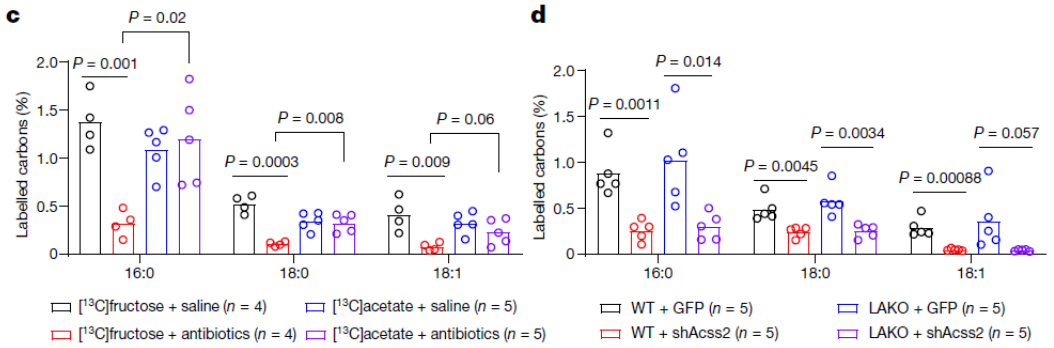


Figura 9. c. Percentuale di carboni totali etichettati in acidi grassi saponificati nel siero di topi LAKO trattati con soluzione salina o antibiotici e a cui è stato somministrato fruttosio:glucosio più 0.5 g/Kg di acetato, con fruttosio o acetato marcati con ^{13}C. **d.** Percentuale di carboni totali etichettati in topi trattati con soluzione 1:1 di fruttosio [^{13}C] e glucosio non marcato, dopo una settimana dall'iniezione di AAV8-GFP (hairpin che non ha alcun effetto sui geni di interesse) o AAV8-shAcss2 (che sopprime l'espressione di ACSS2).

Tuttavia, bisogna considerare che la produzione microbica di acetato dal fruttosio si verifica solo quando il tasso di ingestione dello zucchero supera la capacità di assorbimento dell'intestino tenue. Pertanto, se il fruttosio viene consumato gradualmente, il suo contributo alla DNL potrebbe avvenire in misura maggiore tramite la conversione in acetil-CoA del citrato prodotto dalla fruttolisi epatica (ovvero tramite ACLY), piuttosto che attraverso la produzione di acetato da parte dei microbi (cioè tramite ACSS2). Per esplorare in dettaglio questo aspetto, ai topi è stata somministrata una soluzione di fruttosio:glucosio (con uno o l'altro zucchero marcato con ^{13}C) per 24 ore invece che per 4 o 18 settimane come negli esperimenti precedenti; dopodiché è stata misurata la percentuale di etichettatura degli acidi grassi nel fegato. In questo contesto, in carenza di ACLY (nei topi LAKO) l'etichettatura degli acidi grassi da fruttosio [^{13}C] e glucosio [^{13}C] è ridotta rispetto alla situazione wild-type, come si può vedere nel *in figura 10b* (in ogni caso, dal glucosio deriva solo una piccola parte dei carboni utilizzati in DNL). Tuttavia, se invece che etichettare gli zuccheri direttamente si va a marcare l'acqua (deuterata),

si misura una DNL totale che non differisce notevolmente tra genotipi (vedi *figura 10c*), indicando una disponibilità sufficiente nei topi LAKO di altri intermedi che possono contribuire ai pool lipogenici di acetil-CoA nel fegato. Infatti, l'etichettatura con D_2O va a marcare le molecole a livello degli idrogeni (^2H), non degli atomi di carbonio, e non consente perciò di distinguere da quali substrati iniziali derivino le catene carboniose degli acidi grassi.

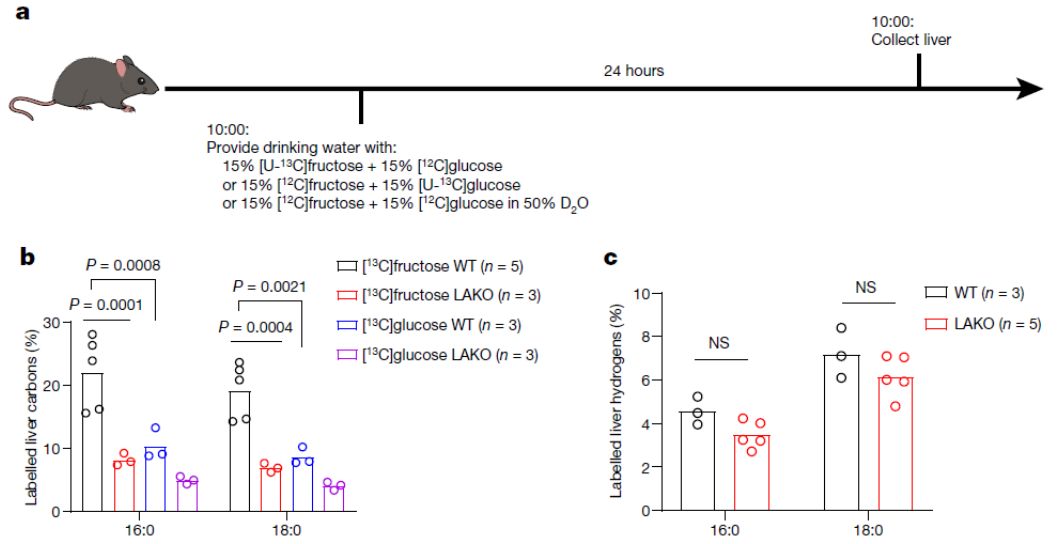


Figura 10. a. Disegno sperimentale per il consumo graduale di fruttosio. [U-^{13}C] = atomi di ^{13}C distribuiti uniformemente nella molecola. **b.** Percentuale di carboni totali etichettati da fruttosio [^{13}C] o glucosio [^{13}C] negli acidi grassi saponificati del fegato di topi WT o LAKO. **c.** Percentuale di idrogeni totali etichettati da D_2O negli acidi grassi saponificati epatici.

CONCLUSIONE

La ricerca di Zhao e coautori dimostra che il consumo di fruttosio incrementa la DNL nel fegato indipendentemente dall'ACLY epatico; tale lipogenesi è sostenuta dal catabolismo del fruttosio da parte del microbiota intestinale in acetato, che raggiunge poi il fegato tramite la vena porta e viene trasformato in acetil-CoA da ACSS2. Tuttavia, l'induzione del programma trascrizionale della DNL epatica sembra essere indipendente sia dall'ACLY che dal microbiota, in quando sarebbe attivata dai metaboliti esosi fosfati derivati da fruttolisi e glicolisi, importanti per l'induzione di ChREBP, un fattore di trascrizione master regolatorio che controlla l'espressione di numerosi geni legati al metabolismo cellulare. Quindi, si propone un modello dell'induzione della DNL dipendente dal catabolismo diretto del fruttosio in cui il metabolismo epatico dello zucchero fornisce un segnale per promuovere la lipogenesi a livello trascrizionale (tramite attivazione di ChREBP), mentre il metabolismo microbico del fruttosio fornisce acetato che le cellule epatiche sono in grado di utilizzare per alimentare la DNL stessa. Questo sistema a doppio meccanismo può anche spiegare il maggiore potenziale lipogenico del fruttosio rispetto al glucosio in un contesto di consumo di grandi quantità di zuccheri, poiché, mentre l'intestino tenue assorbe rapidamente anche masse considerevoli di glucosio, ciò non avviene per il fruttosio, il cui surplus viene trasformato in acetato dal microbiota intestinale.

I dati attuali mostrano un'interazione precedentemente non apprezzata tra dieta, microbiota intestinale e metabolismo degli organi dell'ospite, tutti fattori che possono avere un ruolo importante nell'indurre la steatosi epatica non alcolica stimolata dal fruttosio. L'identificazione dei meccanismi che guidano la DNL e la sua relazione con le malattie metaboliche fornisce una base solida per l'elaborazione di interventi terapeutici mirati. Comprendere come la DNL sia coinvolta nelle patologie può infatti suggerire nuove vie metaboliche o bersagli molecolari che possono essere sfruttati per sviluppare trattamenti innovativi.

BIBLIOGRAFIA

1. Taskinen MR, Packard CJ, Borén J. **Dietary Fructose and the Metabolic Syndrome**. Nutrients. 2019 Aug 22;11(9):1987. doi: 10.3390/nu11091987. PMID: 31443567; PMCID: PMC6770027.
2. Younossi ZM, Golabi P, Paik JM, Henry A, Van Dongen C, Henry L. **The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review**. Hepatology. 2023 Apr 1;77(4):1335-1347. doi: 10.1097/HEP.0000000000000004. Epub 2023 Jan 3. PMID: 36626630; PMCID: PMC10026948.
3. Helsley RN, Moreau F, Gupta MK, Radulescu A, DeBosch B, Softic S. **Tissue-Specific Fructose Metabolism in Obesity and Diabetes**. Curr Diab Rep. 2020 Oct 15;20(11):64. doi: 10.1007/s11892-020-01342-8. PMID: 33057854; PMCID: PMC10208418.
4. Softic S, Cohen DE, Kahn CR. **Role of Dietary Fructose and Hepatic De Novo Lipogenesis in Fatty Liver Disease**. Dig Dis Sci. 2016 May;61(5):1282-93. doi: 10.1007/s10620-016-4054-0. Epub 2016 Feb 8. PMID: 26856717; PMCID: PMC4838515.
5. Sanders FW, Griffin JL. **De novo lipogenesis in the liver in health and disease: more than just a shunting yard for glucose**. Biol Rev Camb Philos Soc. 2016 May;91(2):452-68. doi: 10.1111/brv.12178. Epub 2015 Mar 4. PMID: 25740151; PMCID: PMC4832395.
6. Bose S, Ramesh V, Locasale JW. **Acetate Metabolism in Physiology, Cancer, and Beyond**. Trends Cell Biol. 2019 Sep;29(9):695-703. doi: 10.1016/j.tcb.2019.05.005. Epub 2019 May 31. PMID: 31160120; PMCID: PMC6699882.
7. Mahmood T, Yang PC. **Western blot: technique, theory, and trouble shooting**. N Am J Med Sci. 2012 Sep;4(9):429-34. doi: 10.4103/1947-2714.100998. PMID: 23050259; PMCID: PMC3456489.
8. Caballero-Solares A, Hall JR, Xue X, Rise ML. **Reverse Transcription-Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR) for Gene Expression Analyses**. Methods Mol Biol. 2022;2508:319-340. doi: 10.1007/978-1-0716-2376-3_21. PMID: 35737248.
9. Belew GD, Jones JG. **De novo lipogenesis in non-alcoholic fatty liver disease: Quantification with stable isotope tracers**. Eur J Clin Invest. 2022 Mar;52(3):e13733. doi: 10.1111/eci.13733. Epub 2022 Jan 4. PMID: 34927251.
10. Kailasam S. **LC-MS-What Is LC-MS, LC-MS Analysis and LC-MS/MS**. Technology Networks. 2021 May 17.

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Consumption of fructose has risen markedly in recent decades owing to the use of sucrose and high-fructose corn syrup in beverages and processed foods[1], and this has contributed to increasing rates of obesity and non-alcoholic fatty liver disease[2–4]. Fructose intake triggers de novo lipogenesis in the liver[4–6], in which carbon precursors of acetyl-CoA are converted into fatty acids. The ATP citrate lyase (ACLY) enzyme cleaves cytosolic citrate to generate acetyl-CoA, and is upregulated after consumption of carbohydrates[7]. Clinical trials are currently pursuing the inhibition of ACLY as a treatment for metabolic diseases[8]. However, the route from dietary fructose to hepatic acetyl-CoA and lipids remains unknown. Here, using in vivo isotope tracing, we show that liver-specific deletion of *Acly* in mice is unable to suppress fructose-induced lipogenesis. Dietary fructose is converted to acetate by the gut microbiota[9], and this supplies lipogenic acetyl-CoA independently of ACLY[10]. Depletion of the microbiota or silencing of hepatic ACSS2, which generates acetyl-CoA from acetate, potently suppresses the conversion of bolus fructose into hepatic acetyl-CoA and fatty acids. When fructose is consumed more gradually to facilitate its absorption in the small intestine, both citrate cleavage in hepatocytes and microorganism-derived acetate contribute to lipogenesis. By contrast, the lipogenic transcriptional program is activated in response to fructose in a manner that is independent of acetyl-CoA metabolism. These data reveal a two-pronged mechanism that regulates hepatic lipogenesis, in which fructolysis within hepatocytes provides a signal to promote the expression of lipogenic genes, and the generation of microbial acetate feeds lipogenic pools of acetyl-CoA.

Because ACLY links carbohydrate and lipid metabolism (Extended Data Fig. 1a), we hypothesized that genetic deletion of *Acly* in hepatocytes would protect mice against fructose-induced accumulation of lipids. Although whole-body *Acly* knockout is embryonically lethal[11], mice with liver-specific *Acly*-knockout (LAKO) were grossly indistinguishable from wild-type littermate controls, with similar body weights and organ sizes between genotypes on a diet of either standard chow or high fructose (60%) (Extended Data Fig. 1b, c). Fructose consumption triggered mild hepatic accumulation of lipids, without fibrosis or excess accumulation of glycogen, in both wild-type and LAKO mice (Fig. 1a, Extended Data Fig. 1d, e). Metabolomic and lipidomic analyses revealed notable diet-dependent changes and relatively modest genotype-dependent differences (Extended Data Figs. 2a, b, 3a–c, Supplementary Tables 1, 2). Consistent with loss of ACLY activity, the accumulation of citrate and its downstream metabolite aconitate was observed in livers from LAKO mice (Extended Data Fig. 2c). These data demonstrate that ACLY deficiency, unexpectedly, does not markedly affect global levels of hepatic metabolites or prevent fructose-induced accumulation of triglycerides.

Fructose-driven lipogenesis is ACLY-independent

To further investigate the role of hepatic ACLY in fructose-induced steatosis without altering the overall diet, we fed mice standard chow diets with either normal drinking water or drinking water containing a 1:1 mixture of fructose and glucose (15% each; fructose:glucose) (Extended Data Fig. 4a–c). Similar to mice fed a high-fructose diet, wild-type and LAKO mice drinking fructose:glucose for 4 weeks developed

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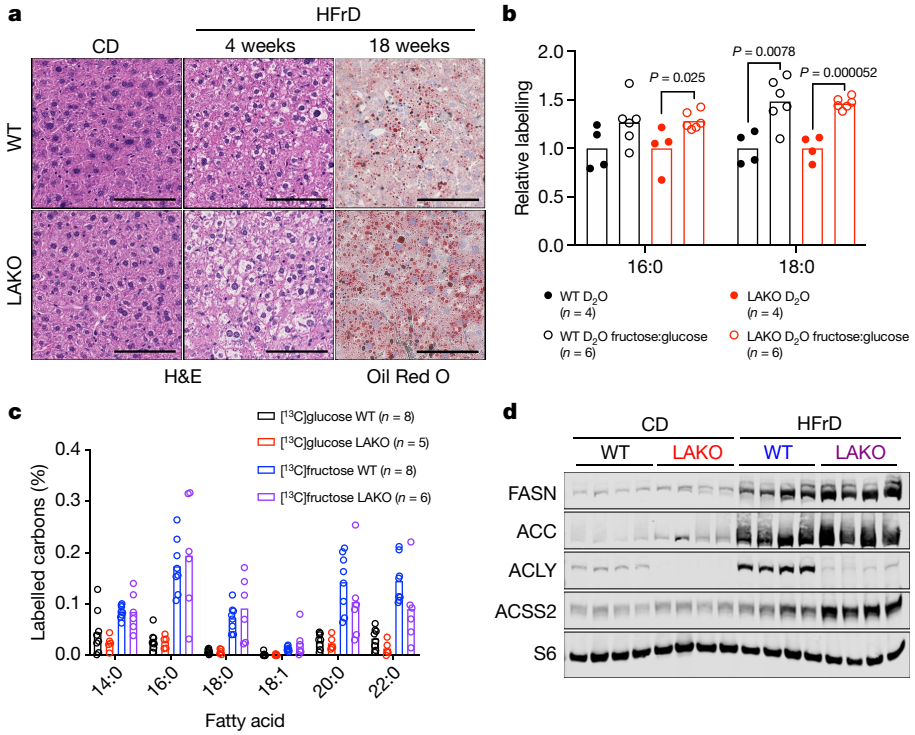

Fig. 1 | Fructose-dependent fatty acid synthesis is ACLY-independent. **a**, Representative haematoxylin and eosin (H&E) and Oil Red O histological stains of livers from wild-type (WT) or LAKO mice fed a chow diet (CD) or a high-fructose diet (HFrD) for 4 or 18 weeks. Images are from two independent experiments ($n = 4$ WT and LAKO mice per diet at 4 weeks; and $n = 13$ WT and $n = 6$ LAKO mice per diet at 18 weeks). Scale bars, 100 µm. **b**, Relative deuterium incorporation in saponified palmitic acid (16:0) and stearic acid (18:0) in liver after D_2O labelling of mice for 24 h. The basal level of D_2O labelling is set to 1 and compared with D_2O labelling after consumption of fructose:glucose within each genotype. *P* values determined by two-sided *t*-tests. **c**, Percentage of total labelled carbons in saponified fatty acids in serum from mice gavaged with 1:1 fructose:glucose, 1.0 g kg^{-1} of each. ^{13}C-labelled substrates are indicated. Data are mean values. **d**, Western blots of lipogenic enzymes in liver lysates of wild-type or LAKO mice fed a chow or high-fructose diet for 4 weeks. Ribosomal protein S6 was used as a loading control. Data in **c**, **d** are representative of two independent experiments.

mild hepatic steatosis (Extended Data Fig. 4d). Moreover, deuterated-water (D_2O) tracing revealed that consumption of fructose:glucose increases hepatic de novo lipogenesis (DNL) to a similar extent in wild-type and LAKO mice (Fig. 1b). Thus, the deletion of *Acly* from liver does not prevent DNL in response to fructose consumption.

Given this unexpected result, we directly tested the effect of ACLY deficiency on fructose conversion into nascent fatty acids. Wild-type and LAKO mice were gavaged with 1:1 fructose:glucose, with either glucose or fructose ^{13}C-labelled (Extended Data Fig. 4e). Notably, fructose carbons were incorporated into fatty acids in LAKO and wild-type mice to a similar extent, whereas glucose carbons were barely used (Fig. 1c, Extended Data Fig. 4f). These data indicate that, in contrast to existing models of fructose metabolism, the use of fructose carbons for hepatic DNL does not require ACLY.

Microbiota-derived acetate feeds lipogenesis

We next investigated the mechanisms of how fructose carbons are used for fatty acid synthesis in an ACLY-independent manner. It has been previously shown that the hepatic DNL program is activated in response to carbohydrate consumption by the ChREBP transcription factor[12,13]. After chronic high-fructose consumption, the livers of both wild-type and LAKO mice upregulated the highly active ChREBP-β isoform[14], along with lipogenic genes (*Acaca* and *Fasn*) and other ChREBP target genes, aldolase B (*Aldob*), and ketohexokinase (*Khk*)[15] (Extended Data Fig. 5a, b). Wild-type mice on a high-fructose diet also exhibited upregulation of *Acly* (Extended Data Fig. 5a). The induction of the DNL program was also robust at the protein level (Fig. 1d, Extended Data Fig. 5c). The residual ACLY protein in livers from fructose-fed LAKO mice was detected in cells other than hepatocytes (Extended Data Fig. 5d). Acyl-CoA synthetase short chain family member 2 (ACSS2), which converts acetate into acetyl-CoA, was notably upregulated in fructose-consuming LAKO mice (Fig. 1d, Extended Data Fig. 5b, c). Moreover, the *Acss2* genomic locus showed increased histone H3K27 acetylation after fructose:glucose drinking (Extended Data Fig. 5e). ChREBP binding to the *Acss2* locus was identified in a published chromatin immunoprecipitation with high-throughput sequencing (ChIP–seq) dataset[16] (Extended Data Fig. 5f). *Acss2* is also a target of SREBP transcription factors, which are activated in response to fructose consumption[17–19]. These data suggest that ACSS2 is a component of the hepatic response to fructose consumption.

Because the conversion of acetate to acetyl-CoA by ACSS2 can support DNL in the absence of ACLY[10], we proposed that acetate might be an important source of acetyl-CoA for DNL in the context of fructose feeding (Fig. 2a). Acetate can be generated within mammalian cells by several mechanisms[20–22], prompting us to investigate whether fructose is converted to acetate in a cell-autonomous manner in hepatocytes. Incubation of mouse hepatocytes with 25 mM [^{13}C]fructose labelled fructolytic intermediates (Fig. 2b), but only minimally labelled acetyl-CoA and malonyl-CoA—the core DNL substrates (Fig. 2c). By contrast, 1 mM [^{13}C]acetate was readily used for the synthesis of acetyl-CoA and malonyl-CoA, as well as HMG-CoA—an intermediate in the mevalonate pathway downstream of acetyl-CoA (Fig. 2c). Therefore, even when ACLY is intact, exogenous acetate directly feeds into lipogenic acetyl-CoA pools in hepatocytes.

We thus investigated the possibility that fructose is converted to acetate before reaching the liver to feed hepatic DNL by performing a [^{13}C]fructose isotope-tracing time-course analysis in mice. Oral administration of [^{13}C]fructose labelled both fructose-1-phosphate (F1P) and

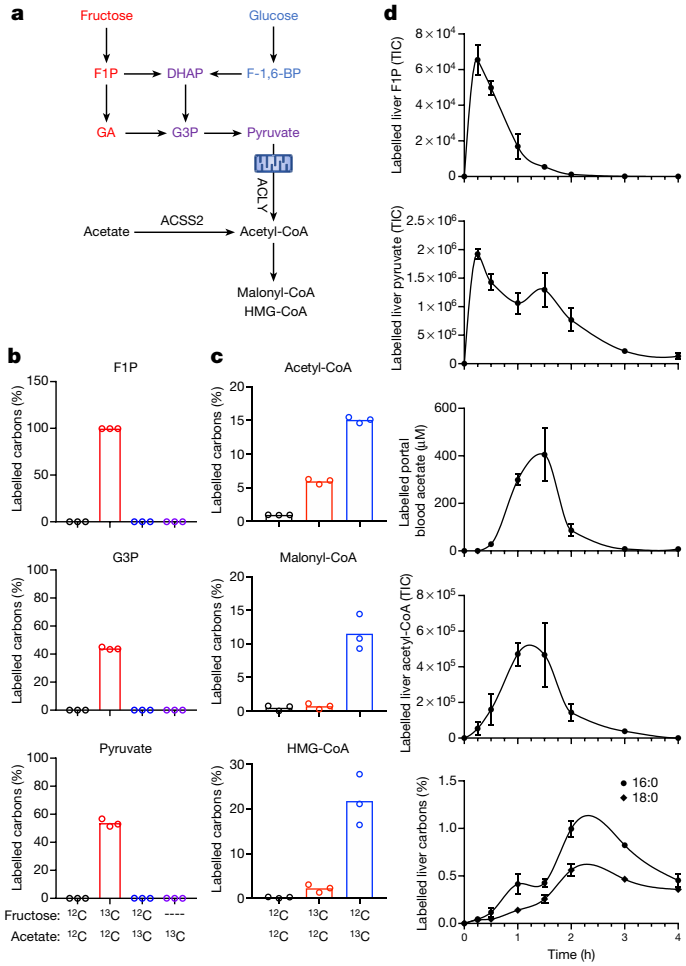

Fig. 2 | Lipogenic acetyl-CoA is preferentially produced from acetate in hepatocytes. a, Pathways for lipogenic acetyl-CoA production from fructose, glucose or acetate. F1P, fructose-1-phosphate; DHAP, dihydroxyacetone phosphate; F-1,6-BP, fructose-1,6-bisphosphate; G3P, glyceraldehyde-3-phosphate; GA, glyceraldehyde. **b**, **c**, Percentage of total labelled carbons in fructolytic intermediates (**b**) and acetyl-CoA, malonyl-CoA or HMG-CoA (**c**) in primary hepatocytes incubated for 6 h with 25 mM fructose plus 1 mM acetate. ^{13}C-labelled substrates are indicated. Data are mean values. $n = 3$ plates of cells, analysed in parallel. **d**, Total ion counts (TIC) of labelled F1P, pyruvate and acetyl-CoA in liver, concentrations of labelled acetate in the portal blood, and the percentage labelling of saponified palmitic acid (16:0) and stearic acid (18:0) in liver of saline-treated wild-type mice gavaged with 1:1 [^{13}C]fructose: unlabelled glucose. Data are mean ± s.e.m. ($n = 3$ mice per time point).

pyruvate in the liver, with peaks between 15 and 30 min after gavage, which indicates rapid absorption of fructose and hepatic fructolysis (Fig. 2d). Hepatic labelling of acetyl-CoA was much slower, peaking at 60–90 min (Fig. 2d). The slower kinetics of acetyl-CoA labelling was closely aligned with the appearance of labelled acetate in the portal circulation (Fig. 2d). Labelling of hepatic fatty acids followed that of acetyl-CoA, peaking at 120–180 min (Fig. 2d), consistent with fructose feeding resulting in the indirect production of hepatic acetyl-CoA and fatty acids via acetate.

We next sought to determine the source of fructose-derived acetate. Although fructose is mainly taken up by the small intestine, unabsorbed fructose reaches the colon where the microbiota converts it into short-chain fatty acids, including acetate[9]. To test whether the microbiota is important for hepatic DNL, we depleted it with an antibiotic cocktail (Extended Data Fig. 6a–d). Antibiotic treatment slightly enhanced the appearance of labelled fructose and fructose-derived glucose in the portal vein after oral administration of [^{13}C]fructose (Extended Data Fig. 6e, f). The induction of hepatic DNL genes after fructose consumption is thought to be dependent on fructolytic and/or glycolytic intermediates[12,23]. Consistent with the normal passage of fructose from the intestine to the liver, DNL gene expression remained intact after antibiotic treatment (Extended Data Fig. 6g), as did the labelling of F1P, pyruvate and citrate in the liver (Fig. 3a, Extended Data Fig. 7a). By contrast, microbiome depletion markedly reduced the labelling from [^{13}C]fructose of hepatic acetyl-CoA and palmitate, as well as fatty acids within circulating lipids (Fig. 3a, b, Extended Data Fig. 7a, b). This reduction was well matched with depleted portal and caecal labelling of acetate and other short-chain fatty acids (Fig. 3a, Extended Data Figs. 7a, 8a, b). Antibiotic treatment also reduced levels of total hepatic triglycerides (Extended Data Fig. 8c), consistent with previous observations[24,25]. Thus, depletion of microbiota suppresses hepatic DNL from [^{13}C]fructose, without impairing fructose metabolism in the small intestine or liver, or the induction of DNL gene expression.

We next investigated whether acetate is a key microbial product supporting DNL. To assess whether fructose intake led to an appreciable increase in concentrations of portal acetate, we measured acetate in portal and systemic serum after gavage. Acetate concentrations in the portal vein increased approximately twofold compared with the baseline (to more than 1 mM) at 60–90 min after fructose gavage (Extended Data Fig. 8d), corresponding to acetate labelling from fructose (Fig. 2d). The rise in portal acetate was absent in antibiotic-treated mice (Extended Data Fig. 8d). Acetate concentrations in the systemic circulation were lower than that in the portal vein and did not markedly fluctuate after fructose consumption, which suggests clearance by the liver (Extended Data Fig. 8d). Next, to assess whether acetate supports DNL downstream of microbial metabolism, mice were gavaged with [^{13}C]acetate, along with 1:1 fructose:glucose. DNL from [^{13}C]acetate, in contrast to that from [^{13}C]fructose, was not affected by antibiotic treatment (Fig. 3c). Finally, to test whether hepatic ACSS2 is required for fructose to feed DNL, ACSS2 in the liver was silenced using an adeno-associated viral (AAV) hairpin targeting *Acss2*[26] (Extended Data Fig. 8e–g). Depletion of hepatic ACSS2 strongly suppressed the labelling of fatty acids in circulating lipids from [^{13}C]fructose (Fig. 3d). Together, these data point to a two-pronged mechanism of DNL after consumption of a fructose bolus, in which sugar metabolism in hepatocytes triggers the DNL transcriptional program, but microbiome-dependent acetate production serves as the major fructose-derived lipogenic substrate, after conversion to acetyl-CoA by hepatic ACSS2 (Extended Data Fig. 10a).

Distinct lipogenic signal and substrates

Microbiota-dependent acetate production from fructose occurs when the rate of ingestion exceeds the uptake capacity of the small intestine[9]. Thus, if fructose is consumed gradually, its contribution to DNL might occur to a greater extent via ACLY, and to a lesser extent via microbial acetate production. Still, after providing fructose:glucose in the drinking water, DNL was comparably stimulated in the presence or absence of ACLY (Fig. 1b). To explore this further, mice were given ^{13}C-labelled fructose or glucose in drinking water for 24 h (Fig. 4a). Fructose-derived carbons provided a substantial contribution to hepatic lipid pools, with greater than 20% of total liver fatty acid carbons being labelled by [^{13}C]fructose after 24 h of fructose:glucose drinking, whereas [^{13}C]glucose contributed less (Fig. 4b). In this context, ACLY deficiency reduced [^{13}C]fructose and [^{13}C]glucose labelling of fatty acids (Fig. 4b). Nevertheless, total DNL as measured by D_2O labelling was not different between genotypes (Fig. 4c), indicating sufficient availability of other two-carbon donors. We hypothesized that acetate from other sources (for example, fibre fermentation) might be assimilated. To test this, we supplemented fructose:glucose drinking water with [^{13}C]acetate at initial exposure and after 2 weeks on fructose:glucose water (Extended Data Fig. 9a). The labelling of fatty acids from [^{13}C]acetate was higher in LAKO mice at baseline (Fig. 4d). After

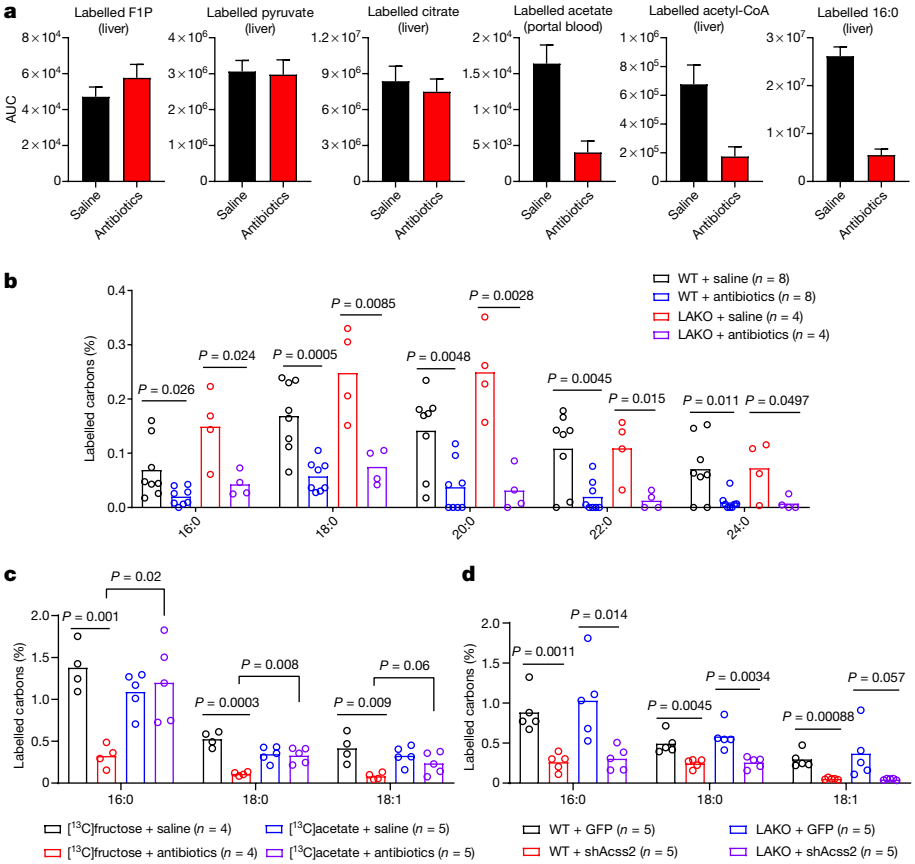

Fig. 3 | Metabolism of bolus fructose by the microbiota feeds hepatic lipogenesis. a, Area under curve ($AUC_{0–240\ min}$) analysis of labelled F1P, pyruvate, citrate, acetyl-CoA and saponified palmitate (16:0) in liver and acetate in portal blood from wild-type mice treated with saline or antibiotics and gavaged with 1:1 [^{13}C]fructose:unlabelled glucose. Data are mean ± s.e.m. See Extended Data Fig. 7a for curves. **b**, Percentage of total labelled carbons in saponified fatty acids in serum from mice treated with saline or antibiotics and gavaged with 1:1 [^{13}C]fructose:unlabelled glucose. *P* values determined by two-sided *t*-tests. **c**, Percentage of total labelled carbons in saponified fatty acids in serum from LAKO mice treated with saline or antibiotics and gavaged with 1:1 fructose:glucose plus 0.5 g kg^{-1} acetate. ^{13}C-labelled substrates are indicated. *P* values determined by two-sided *t*-tests. **d**, Percentage of total labelled carbons in saponified fatty acids in serum from 1:1 [^{13}C]fructose:unlabelled glucose in wild-type and LAKO mice 1 week after tail vein injection with AAV8-GFP or AAV8-shAcss2. *P* values determined by two-sided *t*-tests. Data in **b**–**d** denote mean values.

fructose conditioning, the contribution of acetate to DNL increased in wild-type mice, and this was further enhanced in LAKO mice (Fig. 4d), consistent with increased hepatic ACSS2 expression in LAKO mice after fructose feeding, which preceded the onset of steatosis (Extended Data Fig. 9b, c). We next assessed the contribution of microbiome-derived acetate from all dietary sources in the context of gradual fructose consumption. Antibiotic treatment suppressed total hepatic DNL in LAKO mice (Fig. 4e, Extended Data Fig. 9d). ChREBP-β and DNL gene expression were confirmed to be upregulated by fructose:glucose drinking in all groups, indicating that their regulation in response to fructose consumption is independent of acetyl-CoA metabolism (Fig. 4f). Finally, we examined DNL in mice given fructose:glucose after the silencing of hepatic ACSS2, and found that in the context of gradual fructose consumption via drinking water, the loss of both ACLY and ACSS2 is necessary to suppress DNL (Fig. 4g). These data indicate that when fructose is consumed gradually to facilitate its absorption in the small intestine, the rate of DNL is established by signalling mechanisms (that is, sugar-driven activation of ChREBP), and DNL is suppressed only when acetyl-CoA production by both ACLY and ACSS2 is inhibited (Extended Data Fig. 10b).

Discussion

This study demonstrates that bolus fructose consumption triggers hepatic DNL that is independent of liver ACLY but dependent on the metabolism of fructose by the gut microbiota to acetate, which then reaches the liver via the portal vein. The induction of the DNL transcriptional program in the liver, however, seems to be independent of both ACLY and the microbiome, consistent with evidence that hexose phosphate metabolites are important for ChREBP activation[19,27]. This may explain why *Khk*-knockout mice are protected from fructose-induced fatty liver[28,29]. Thus, we propose a revised model of fructose-dependent DNL induction, in which hepatic fructose metabolism provides a signal to promote DNL transcriptionally while microbial fructose metabolism provides acetate to feed DNL (Extended Data Fig. 10a). These dual mechanisms may also explain the higher lipogenic potential of fructose as compared to glucose[30], at least in the context of high-dose sugar consumption, in that the small intestine rapidly absorbs even large loads of glucose, whereas fructose reaches the gut microbiota, which generate acetate[9]. When consumed more gradually, fructose can feed DNL in an ACLY-dependent manner. However, acetate from other sources is also readily available to the liver, rendering ACLY dispensable for DNL even when fructose is gradually consumed (Extended Data Fig. 10b). Of note, acetate is probably insufficient to trigger an increase in DNL in the absence of the sugar-derived lipogenic signal. Thus, it will be important to define how fructose interacts with dietary sources of acetate such as ethanol and fermentable fibres.

Understanding the fundamental pathways involved in hepatic DNL is important for the development of therapeutic interventions

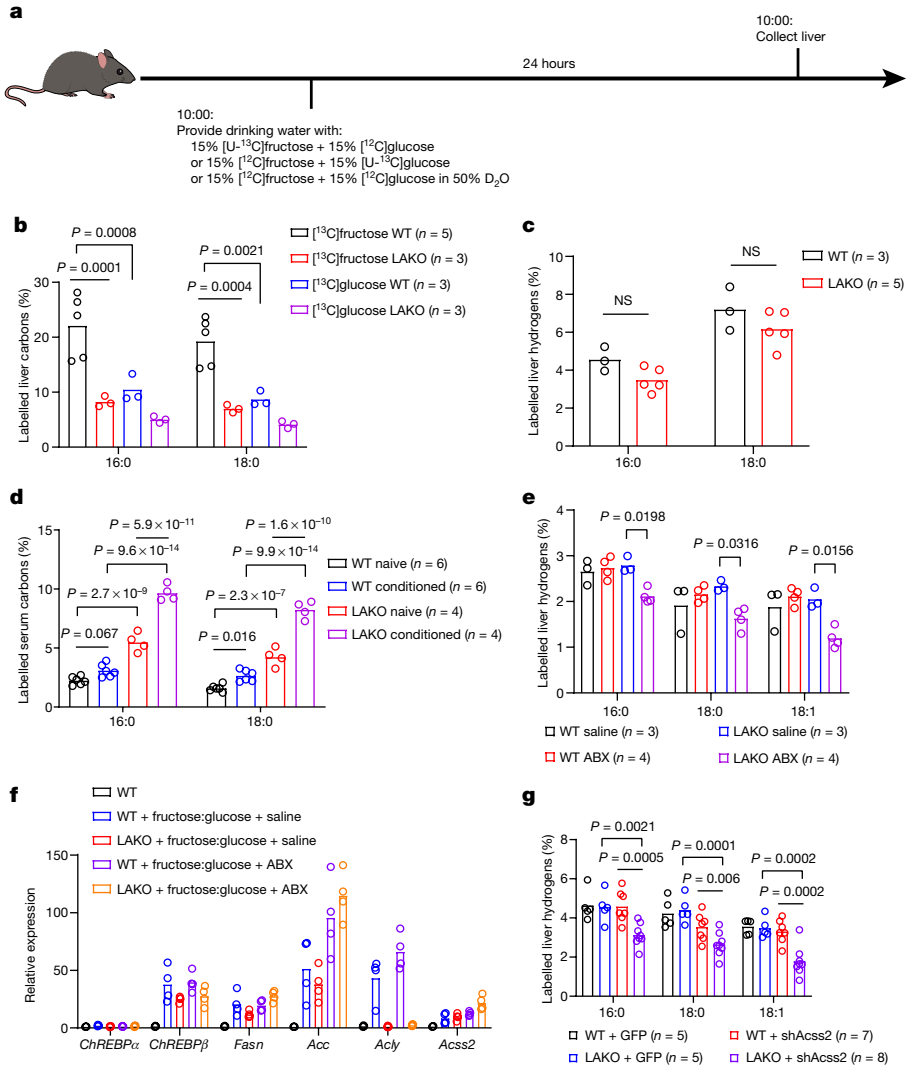

Fig. 4 | Gradual consumption of fructose promotes hepatic lipogenesis from ACLY- and ACSS2-derived acetyl-CoA. a, Experimental design for gradual fructose consumption. [U-^{13}C] denotes uniformly labelled ^{13}C. **b**, Percentage of total labelled carbons from [^{13}C]fructose or [^{13}C]glucose in saponified hepatic fatty acids from wild-type or LAKO mice. **c**, Percentage of total labelled hydrogens from D_2O in saponified hepatic fatty acids. **d**, Percentage of total labelled carbons from [^{13}C]acetate in saponified fatty acids in serum. See Extended Data Fig. 9a for experimental design. **e**, Percentage of total labelled hydrogens from D_2O in saponified hepatic fatty acids from wild-type and LAKO mice after treatment with saline or antibiotics (ABX) for 1 week. **f**, mRNA expression of *ChREBP* (also known as *Mlxipl*) and lipogenic genes in the livers of mice in **e**. **g**, Percentage of total hydrogens labelled in saponified hepatic fatty acids from wild-type and LAKO mice 1 week after injection with AAV8-GFP or AAV8-shAcss2. Data in **b**–**g** denote mean values. All *P* values determined by two-way analysis of variance (ANOVA) with Tukey's test for multiple comparisons.

for metabolic diseases. In addition to acetate, the microbiome also produces other short-chain fatty acids such as butyrate and propionate. Butyrate has been shown to contribute to hepatic lipogenesis[31], presumably by first entering the mitochondrial TCA cycle and then feeding extra-mitochondrial pools of acetyl-CoA in an ACLY-dependent manner[32]. Diet and microbiome could potentially affect the efficacy of ACLY inhibitors, which are currently in clinical trials for hypercholesterolaemia[33]. Previous studies of RNA-interference-mediated ACLY deficiency report decreased hepatic lipids in *db/db* mice and mice fed a high-fat, high-sucrose diet, but increased hepatic lipids in mice fed a high-fat diet only[34–36]. In our own data, principal component analysis of hepatic triglycerides separated LAKO mice from wild-type mice on a high-fructose but not a chow diet (Extended Data Fig. 3c), supporting the notion that ACLY may have distinct roles depending on diet. The current data determine a previously unappreciated interaction between diet, the gut microbiome, and host organ metabolism that contributes to fructose-induced non-alcoholic fatty liver disease.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-2101-7.

1. Bray, G. A., Nielsen, S. J. & Popkin, B. M. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *Am. J. Clin. Nutr.* **79**, 537–543 (2004).
2. Jensen, T. et al. Fructose and sugar: A major mediator of non-alcoholic fatty liver disease. *J. Hepatol.* **68**, 1063–1075 (2018).
3. Hannou, S. A., Haslam, D. E., McKeown, N. M. & Herman, M. A. Fructose metabolism and metabolic disease. *J. Clin. Invest.* **128**, 545–555 (2018).
4. Softic, S., Cohen, D. E. & Kahn, C. R. Role of dietary fructose and hepatic de novo lipogenesis in fatty liver disease. *Dig. Dis. Sci.* **61**, 1282–1293 (2016).
5. Lambert, J. E., Ramos-Roman, M. A., Browning, J. D. & Parks, E. J. Increased de novo lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease. *Gastroenterology* **146**, 726–735 (2014).
6. Donnelly, K. L. et al. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J. Clin. Invest.* **115**, 1343–1351 (2005).

7. Fukuda, H., Katsurada, A. & Iritani, N. Effects of nutrients and hormones on gene expression of ATP citrate-lyase in rat liver. *Eur. J. Biochem*. **209**, 217–222 (1992).
8. Pinkosky, S. L., Groot, P. H. E., Lalwani, N. D. & Steinberg, G. R. Targeting ATP-Citrate Lyase in Hyperlipidemia and Metabolic Disorders. *Trends Mol. Med*. **23**, 1047–1063 (2017).
9. Jang, C. et al. The small intestine converts dietary fructose into glucose and organic acids. *Cell Metab*. **27**, 351–361.e3 (2018).
10. Zhao, S. et al. ATP-citrate lyase controls a glucose-to-acetate metabolic switch. *Cell Rep*. **17**, 1037–1052 (2016).
11. Beigneux, A. P. et al. ATP-citrate lyase deficiency in the mouse. *J. Biol. Chem*. **279**, 9557–9564 (2004).
12. Herman, M. A. & Samuel, V. T. The sweet path to metabolic demise: fructose and lipid synthesis. *Trends Endocrinol. Metab*. **27**, 719–730 (2016).
13. Uyeda, K. & Repa, J. J. Carbohydrate response element binding protein, ChREBP, a transcription factor coupling hepatic glucose utilization and lipid synthesis. *Cell Metab*. **4**, 107–110 (2006).
14. Herman, M. A. et al. A novel ChREBP isoform in adipose tissue regulates systemic glucose metabolism. *Nature* **484**, 333–338 (2012).
15. Iizuka, K. The role of carbohydrate response element binding protein in intestinal and hepatic fructose metabolism. *Nutrients* **9**, 181 (2017).
16. Poungvarin, N. et al. Genome-wide analysis of ChREBP binding sites on male mouse liver and white adipose chromatin. *Endocrinology* **156**, 1982–1994 (2015).
17. Luong, A., Hannah, V. C., Brown, M. S. & Goldstein, J. L. Molecular characterization of human acetyl-CoA synthetase, an enzyme regulated by sterol regulatory element-binding proteins. *J. Biol. Chem*. **275**, 26458–26466 (2000).
18. Ikeda, Y. et al. Transcriptional regulation of the murine acetyl-CoA synthetase 1 gene through multiple clustered binding sites for sterol regulatory element-binding proteins and a single neighboring site for Sp1. *J. Biol. Chem*. **276**, 34259–34269 (2001).
19. Softic, S. et al. Divergent effects of glucose and fructose on hepatic lipogenesis and insulin signaling. *J. Clin. Invest*. **127**, 4059–4074 (2017).
20. Liu, X. et al. Acetate production from glucose and coupling to mitochondrial metabolism in mammals. *Cell* **175**, 502–513.e13 (2018).
21. Bulusu, V. et al. Acetate recapturing by nuclear acetyl-coa synthetase 2 prevents loss of histone acetylation during oxygen and serum limitation. *Cell Rep*. **18**, 647–658 (2017).
22. Lu, M. et al. ACOT12-dependent alteration of acetyl-coa drives hepatocellular carcinoma metastasis by epigenetic induction of epithelial-mesenchymal transition. *Cell Metab*. **29**, 886–900.e5 (2019).
23. Ter Horst, K. W. & Serlie, M. J. Fructose consumption, lipogenesis, and non-alcoholic fatty liver disease. *Nutrients* **9**, 981 (2017).
24. Bergheim, I. et al. Antibiotics protect against fructose-induced hepatic lipid accumulation in mice: role of endotoxin. *J. Hepatol*. **48**, 983–992 (2008).
25. Kaden-Volynets, V. et al. Lack of liver steatosis in germ-free mice following hypercaloric diets. *Eur. J. Nutr*. **58**, 1933–1945 (2019).
26. Mews, P. et al. Acetyl-CoA synthetase regulates histone acetylation and hippocampal memory. *Nature* **546**, 381–386 (2017).
27. Li, M. V. et al. Glucose-6-phosphate mediates activation of the carbohydrate responsive binding protein (ChREBP). *Biochem. Biophys. Res. Commun*. **395**, 395–400 (2010).
28. Lanaspa, M. A. et al. Ketohexokinase C blockade ameliorates fructose-induced metabolic dysfunction in fructose-sensitive mice. *J. Clin. Invest*. **128**, 2226–2238 (2018).
29. Ishimoto, T. et al. Opposing effects of fructokinase C and A isoforms on fructose-induced metabolic syndrome in mice. *Proc. Natl Acad. Sci. USA* **109**, 4320–4325 (2012).
30. Parks, E. J., Skokan, L. E., Timlin, M. T. & Dingfelder, C. S. Dietary sugars stimulate fatty acid synthesis in adults. *J. Nutr*. **138**, 1039–1046 (2008).
31. den Besten, G. et al. Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. *Am. J. Physiol. Gastrointest. Liver Physiol*. **305**, G900–G910 (2013).
32. Donohoe, D. R. et al. The Warburg effect dictates the mechanism of butyrate-mediated histone acetylation and cell proliferation. *Mol. Cell* **48**, 612–626 (2012).
33. Zagelbaum, N. K., Yandrapalli, S., Nabors, C. & Frishman, W. H. Bempedoic acid (ETC-1002): ATP citrate lyase inhibitor: review of a first-in-class medication with potential benefit in statin-refractory cases. *Cardiol. Rev*. **27**, 49–56 (2019).
34. Guo, L. et al. Enhanced acetylation of ATP-citrate lyase promotes the progression of nonalcoholic fatty liver disease. *J. Biol. Chem*. **294**, 11805–11816 (2019).
35. Wang, Q. et al. Abrogation of hepatic ATP-citrate lyase protects against fatty liver and ameliorates hyperglycemia in leptin receptor-deficient mice. *Hepatology* **49**, 1166–1175 (2009).
36. Wang, Q. et al. Deficiency in hepatic ATP-citrate lyase affects VLDL-triglyceride mobilization and liver fatty acid composition in mice. *J. Lipid Res*. **51**, 2516–2526 (2010).

Methods

Data reporting

No statistical methods were used to predetermine sample size. The experiments were not randomized and investigators were not blinded to allocation during experiments and outcome assessment.

Generation of LAKO mice

Generation of *Acly*$^{f/f}$ mice on a C57Bl6/J background was previously described[10]. To generate hepatocyte-specific *Acly* knockouts, *Acly*$^{f/f}$ mice were crossed to albumin-Cre transgenic mice (B6.Cg$^{Tg(Alb\text{-}cre)21Mgn}$/J, Jackson Laboratory)[37].

Genotying

Genotyping of the recombined *Acly* allele was confirmed as previously described[10]. Genotyping of the *Alb-cre* allele was confirmed with the following primer sequences: *Alb-cre*-5′F (CCTGCCAGCATGGATATAA), *Alb-cre*-3′R (GTTGTCCTTTGTGCTGCTGA), *Alb*-TSP3 (GAAGCAGAAGCTTAGGAAGATGG), and the following cycling conditions: 1 cycle at 94 °C for 5 min; 35 cycles at 94 °C for 45 s, 58 °C for 45 s, 72 °C for 1 min; and 1 cycle at 72 °C for 10 min; hold at 4 °C.

Mouse studies

All animal protocols in this study were approved by the University of Pennsylvania's Institutional Animal Care and Use Committee (IACUC) and Princeton University's IACUC. For diet studies, 4-week-old male mice were placed on either a regular chow diet (Lab Diet 5010) or a high-fructose chow diet (Teklad TD.89247) for indicated lengths of time. Weights of mice kept on each diet were taken weekly. For drinking-water studies, mice were provided with regular tap water (filtered through a 0.22-µm filter) or 15% (w/v) fructose:15% (w/v) glucose (Sigma F3510, G8270) in tap water (filtered through a 0.22-µm filter). Daily water consumption was determined by measuring the total consumption in a cage, divided by the number of mice in the cage. To deplete the gut microbiome, mice were given a daily 10 µl g^{-1} body weight oral gavage consisting of 1 mg ml^{-1} ampicillin, 1 mg ml^{-1} gentamicin, 0.5 mg ml^{-1} vancomycin, 1 mg ml^{-1} neomycin, 1 mg ml^{-1} metronidazole in a 0.9% NaCl solution for 7–10 days. Studies were controlled to mice given the same 0.9% NaCl solution without antibiotics. To knockdown *Acss2*, 6–8-week-old male mice were injected via tail-vein with 2.0×10^{11} genome copies per mouse of AAV8.U6.shAcss2.CMV.eGFP.SV40 (University of Pennsylvania Vector Core) or AAV8.CMV.PI.eGFP.WPRE.bGH (Addgene) as a control; experiments were performed 1 week after injection.

Histology

For H&E, Periodic Acid Shiff and trichrome staining: tissues were fixed in formalin overnight, dehydrated by titrating in ethanol (50%, 75% and 95%), and submitted to the Molecular Pathology and Imaging Core at the University of Pennsylvania for paraffin embedding, sectioning and staining. For Oil Red O staining: tissues were fixed in formalin overnight, dehydrated by titrating in sucrose (10%, 20% and 30%), and embedded in Richard-Allan Scientific NEG-50 frozen section medium (ThermoFisher Scientific, 6502) by freezing in 2-methylbutane that was cooled using dry ice. Tissues frozen in NEG-50 were submitted to the Molecular Pathology and Imaging Core at the University of Pennsylvania for cryosectioning and staining. Images were acquired on a Keyence BZ-X710 microscope.

Bacterial quantification

Caecal contents were collected, snap-frozen and weighed before storage at −80 °C until use. DNA was extracted from caecal contents using a Fecal DNA extraction kit (IBI Scientific, IB47821) according to manufacturer's instructions. Samples were diluted 1:1,000 before use for reverse transcription PCR (RT–PCR). To establish a bacterial DNA standard, genomic DNA was extracted from Stbl3 *E. coli* cells. A standard curve was generated using a 1:4 serial dilution starting with 10 ng of *E. coli* DNA. RT–PCR was performed as described, using previously published universal *16S* primers (forward: 5′-TCCTACGGGAGGCAGCAGT-3′, reverse: 5′-GGACTACCAGGGTATCTAATCCTGTT-3′)[38]. Relative bacterial load was calculated by normalizing DNA content to initial caecal content weight.

Western blotting

Protein extraction from tissue was performed by re-suspending frozen tissue in 0.5 ml of RIPA buffer (1% NP-40, 0.5% deoxycholate, 0.1% SDS, 150 mM NaCl, 50 mM Tris plus protease and phosphatase inhibitors), and lysed using a tissue lyser (Qiagen) twice for 30 s at 20 Hz. After lysis, samples were incubated on ice for 10 min, then spun down at 15,000*g* for 5 min at 4 °C. Supernatant was collected and stored in −80 °C until immunoblotting. Antibodies used in this study were: ACLY (Proteintech, 15421-1-AP), ACSS2 (Cell Signaling Technology, 3658S), acetyl-CoA carboxylase (Cell Signaling Technology, 3676S), fatty acid synthase (Cell Signaling Technology 3189S), catalase (Cell Signaling Technology, 14097S), ribosomal protein S6 (Cell Signaling Technology 2217S), and IRDye800CW goat anti-rabbit (LI-COR 926-32211). Immunoblots were developed using a LI-COR Odyssey Clx.

RT–qPCR

RNA extraction from tissue was performed by re-suspending frozen tissue in 1 ml Trizol (Life Technologies), and lysed using a tissue lyser (Qiagen) for 60 s at 30 Hz, followed by manufacturer protocol for Trizol RNA extraction. cDNA was synthesized using high-capacity RNA-to-cDNA master mix (Applied Biosystems, 4368814), as per the kit instructions. cDNA was diluted 1:20 and amplified using PowerUp SYBR Green Master Mix (Applied Biosystems, A25778) on the ViiA-7 Real-Time PCR system. Fold change in expression was calculated using ΔC_t, with the *18S* reference gene as an endogenous control. Primer sequences for RT–qPCR are: *Aldob* (forward: GAAACCGCCTGCAAAGGATAA, reverse: GAGGGTCTCGTGGAAAAGGAT), *Khk* (forward: ATGTGGTGGACAAATACCCAGA, reverse: CAAGCAAGGAAAGGACAGTGC), *Acly* (forward: TTCGTCAAACAGCACTTCC, reverse: ATTTGGCTTCTTGGAGGTG), *Acss2* (forward: GCTTCTTTCCCATTCTTCGGT, reverse: CCCGGACTCATTCAGGATTG), *ChREBPa* (forward: CGACACTCACCCACCTCTTC, reverse: TTGTTCAGCCGGATCTTGTC), *ChREBPb* (forward: TCTGCAGATCGCGTGGAG, reverse: CTTGTCCCGGCATAGCAAC), *Fasn* (forward: ATTGGTGGTGTGGACATGGTC, reverse: CCCAGCCTTCCATCTCCTG), *Acc1* (forward: ACAGTGGAGCTAGAATTGGAC, reverse: ACTTCCCGACCAAGGACTTTG).

Measurement of DNL using isotope tracers

To assess total lipogenesis, mice were provided with 50% (v/v) deuterated water (Sigma 151882) mixed into 15% fructose:15% glucose (w/v) drinking water for 24 h. Systemic blood was collected by cardiac puncture, allowed to coagulate on ice for 15 min, and spun down at 15,000*g* for 10 min at 4 °C to collect serum. To account for differences in drinking water consumption, calculated deuterium enrichment labelling in serum water was used to normalize labelling into fatty acids. To assess lipogenesis from dietary carbohydrates, on the day of experiment, mice were weighed and fasted from 10:00 until 15:00, when they were given an oral gavage consisting of a 1:1 mixture of glucose and fructose in 0.9% NaCl saline. Doses used in this study ranged from 1.0 g kg^{-1} or 2.0 g kg^{-1} of each hexose. [U-^{13}C]glucose (Cambridge Isotope Laboratories, CLM-1396-1) or [U-^{13}C]fructose (Cambridge Isotope Laboratories, CLM-1553-1) were provided with the corresponding unlabelled hexose. Six hours after gavage, systemic blood was collected by tail bleeding the mice and incubating the blood on ice for 15 min before spinning down at 15,000*g* for 10 min at 4 °C to collect serum. To assess lipogenesis from dietary acetate, this procedure was repeated, but with an oral gavage containing 2.0 g kg^{-1} of unlabelled glucose and fructose with 0.5 g kg^{-1} [1,2-^{13}C]acetate

(Cambridge Isotope Laboratories, CLM-440-1). To assess lipogenesis from dietary carbohydrates in drinking water, [U-^{13}C]glucose or [U-^{13}C]fructose were provided with the corresponding unlabeled hexose at 15% (w/v) in drinking water for 24 h. To assess lipogenesis from dietary acetate in drinking water, 150 mM [1,2-^{13}C]acetate was added to 15% fructose:15% glucose (w/v) for 24 h. Tissues were collected using a clamp pre-cooled with liquid nitrogen. The frozen liver samples were ground at liquid nitrogen temperature with a Cryomill (Retsch). Saponification of lipids and liquid chromatography–mass spectrometry (LC–MS) analysis were performed as previously described[39]. In brief, serum (5 µl) or liver powder (10 mg) was incubated with 1 ml of 0.3 M KOH in 90% methanol at 80 °C for 1 h in a 2 ml glass vial. Formic acid (0.1 ml) was then added for neutralization. The saponified fatty acids were extracted by adding 0.5 ml of hexane, vortexing, and transferring the top hexane layer to a new glass vial. Samples were then dried under a stream of nitrogen gas and dissolved in 100 µl (for serum) or 1 ml (for liver) of isopropanol:methanol (1:1; v/v) solution for LC–MS analysis. Separation was performed by reversed-phase ion-pairing chromatography on a C8 column coupled to negative-ion mode, full-scan LC–MS at 1-Hz scan time and 100,000 resolving power (stand-alone orbitrap; Thermo Fischer Scientific). Data analysis with MAVEN software and natural isotope correction were performed as previously described[40].

Primary hepatocyte isolation

Hepatocytes were isolated using a two-step collagenase and DNase digestion protocol[41] and plated in M199 media containing 5 mM glucose, 10% FBS, 500 nM dexamethasone and 1 nM insulin. After attachment, cells were changed to M199 media containing 5 mM glucose, 500 nM dexamethasone and incubated overnight. Cells were switched to M199 containing 5 mM glucose, 10% FBS, 500 nM dexamethasone, 100 nM insulin and respective fructose and acetate supplementation for 6 h on the day of experiment. Evidence that high concentrations of glucose are required to induce the DNL gene program in primary hepatocytes[42] informed our use of 25 mM fructose.

Acyl-CoA measurements in primary hepatocytes

Measurements of Acyl-CoA in primary hepatocytes were performed by LC–MS/high-resolution mass spectrometry as previously described[43]. In brief, primary hepatocytes were isolated and cultured in 6-well plates as described in 'Primary hepatocyte isolation'. At collection, culture medium was completely aspirated before collecting cells in 0.5 ml ice-cold 10% trichloroacetic acid per well of a 6-well dish using a cell lifter. Samples were then sonicated for 10 × 0.5 s pulses to completely disrupt cellular membranes, and incubated on ice to precipitate proteins. Protein was pelleted at 16,000*g* for 10 min at 4 °C. Supernatant was collected and purified by solid-phase extraction using Oasis HLB 1cc (30 mg) SPE columns (Waters). Eluate was evaporated to dryness under nitrogen gas and re-suspended in 50 µl of 5% 5-sulfosalicylic acid (w/v) for injection. Samples were analysed by an Ultimate 3000 autosampler coupled to a Thermo Q-Exactive Plus instrument in positive electrospray ionization (ESI) mode. For isotopic tracer analysis, isotopic enrichment from [U-^{13}C]fructose (Cambridge Isotope Laboratories, CLM-1553) or [U-^{13}C]acetate (Cambridge Isotope Laboratories, CLM-440-1) was calculated to compensate for the nonlinearity of isotopic enrichment using the FluxFix calculator[44].

Fructolyic measurements in primary hepatocytes

For fructolytic intermediate measurements in primary hepatocytes, culture medium was completely aspirated before collecting cells in 0.5 ml of cold 80:20 methanol:water per well of a 6-well dish using a cell lifter. Samples were then sonicated for 10 × 0.5-s pulses to completely disrupt cellular membranes, and incubated on ice. Samples were then spun down at 16,000*g* for 10 min at 4 °C. Supernatant was collected and dried under nitrogen gas flow in preparation for water-soluble metabolomic analysis.

ChIP–qPCR

For H3K27ac ChIP–qPCR studies, wild-type male mice were provided with fructose:glucose drinking water for 24 h, and orally gavaged with 2.0 g kg^{-1} fructose plus 2.0 g kg^{-1} glucose 1 h before being euthanized. ChIP was performed as previously described[45] with adjustments to start from liver tissue. Liver tissues were obtained from mice 90 min after gavage, and 100 mg of tissue was weighed. Tissues were homogenized by mincing briefly with razor blades followed by resuspension in 5 ml of ice-cold PBS and several passages through a 16-gauge syringe needle into 15-ml conical tubes. Samples were crosslinked with 2% formaldehyde for 10 min at room temperature. The reactions were quenched with 0.25 M glycine. The cells were then washed with PBS and resuspended in cell lysis buffer (10 mM Tris-HCl, pH 8.1, 10 mM NaCl, 1.5 mM $MgCl_2$, 0.5% NP-40), supplemented with protease inhibitors (Roche). The cell pellet was resuspended in 0.5 ml of nuclear lysis buffer (50 mM Tris-HClm pH 8.1, 5 mM EDTA, 1% SDS) supplemented with protease inhibitors. The chromatin was fragmented with a Diagenode Bioruptor Pico (12 cycles of 30 s on followed by 30 s off, at 4 °C). Samples were incubated with protein G magnetic beads (Millipore-Sigma, 16-662) and H3K27ac (Abcam, ab4729) or normal rabbit IgG (Cell Signalling Technology, 2729S) antibody overnight at 4 °C. The next day, samples were washed five times with decreasingly stringent buffers. ChIP DNA was eluted off the beads by incubating beads in 125 µl elution buffer for 10 min at 65 °C. The combined supernatant was then incubated overnight at 65 °C to reverse crosslinks and treated with proteinase K for 1 h the next morning. Samples were purified using Macherey-Nagel DNA purification kit, with NTB binding buffer. Samples were diluted 1:5 in nuclease-free water before RT–qPCR reactions, which were performed as described above with the following primers: *Mlxipl* p1 (forward: CGCACCCGGTCTACAGTTT, reverse: GTGCCTCCTTCTCTCCTTAGC), *Mlxipl* p2 (forward: GCCATCCACGTGCTAAGGA, reverse: GGCTTTTAGACTGGGGTGTGG), *Pklr* p1 (forward: GGGAAGGATGCCCACTACAG, reverse: TGGAAGCCTTGTACACTGGG), *Pklr* p2 (forward: CCCAGTGTACAAGGCTTCCAT, reverse: CTCTGCCTTTGTCAGTGGGA), *Acss2* p1 (forward: ATTGGATGCCTAGAGCACGG, reverse: CGCATCAAGTTCCGAACACC), *Acss2* p2 (forward: TCAGGACAGTTTAGGGTGCAA, Reverse: TTACAAAGACCTGCCTCTGCC).

Triglyceride measurements

Triglyceride measurements were performed using a Triglyceride Colorimetric Assay Kit (Cayman Chemical, 10010303) as per manufacturer's instructions.

Metabolomics

Water-soluble metabolite extraction was performed as previously described[9]. For serum samples, 100 µl of methanol at −20 °C was added to 5 µl of serum sample and incubated on ice for 10 min, followed by vortexing and centrifugation at 16,000*g* for 10 min at 4 °C. The supernatant (first extract) was transferred to a new tube. Then, 50 µl methanol was added to resuspend the pellet, followed by vortexing and centrifugation at 16,000*g* for 10 min at 4 °C. The supernatant (second extract) was combined with the first extract. Then, 3 µl of the 150 µl extract was loaded to LC–MS. For tissue samples, frozen tissue samples were ground at liquid nitrogen temperature with a Cryomill (Retsch). The resulting tissue powder was weighed (approximately 20 mg). The extraction was then done by adding −20 °C 40:40:20 methanol:acetonitrile:water to the powder and incubating at −20 °C overnight, followed by vortexing and centrifugation at 16,000*g* for 10 min at 4 °C. The volume of the extraction solution (µl) was 40 × the weight of tissue (mg) to make an extract of 25 mg tissue per millilitre solvent. Serum and tissue extracts were analysed by LC–MS, using two different LC–MS methods chosen for optimal separation of glucose and fructose (in serum) and of hexose phosphate species (from tissues). Serum extracts were analysed (without drying) using a quadrupole-orbitrap mass spectrometer (Q Exactive, Thermo Fisher Scientific)

operating in negative ion mode, coupled to hydrophilic interaction chromatography via electrospray ionization and used to scan from *m/z* 70 to 1000 at 1 Hz and 75,000 resolution. Liquid chromatography separation was on a XBridge BEH Amide column (2.1 mm × 150 mm, 2.5 µm particle size, 130 Å pore size) using a gradient of solvent A (20 mM ammonium acetate, 20 mM ammonium hydroxide in 95:5 water: acetonitrile, pH 9.45) and solvent B (acetonitrile). Flow rate was 150 µl min^{-1}. The liquid chromatography gradient was: 0 min, 85% B; 2 min, 85% B; 3 min, 80% B; 5 min, 80% B; 6 min, 75% B; 7 min, 75% B; 8 min, 70% B; 9 min, 70% B; 10 min, 50% B; 12 min, 50% B; 13 min, 25% B; 16 min, 25% B; 18 min, 0% B; 23 min, 0% B; 24 min, 85% B; 30 min, 85% B. Autosampler temperature was 5 °C, and injection volume was 3 µl. Tissue extracts were dried under nitrogen gas flow and re-dissolved in LC–MS grade water. Metabolites were analysed via reverse-phase ion-pairing chromatography coupled to an Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific). The mass spectrometer was operated in negative ion mode with resolving power of 100,000 at *m/z* 200 and scan range of *m/z* 75–1000. The liquid chromatography method was modified from an earlier method[46], using an Atlantis T3 column (150 mm × 2.1 mm, 3 µm particle size, 100 Å pore size), with a gradient of solvent A (97:3 water:methanol with 10 mM tributylamine and 15 mM acetic acid) and solvent B (methanol). The liquid chromatography gradient was 0 min, 0% B, 200 µl min^{-1}; 2 min, 0% B, 200 µl min^{-1}; 4 min, 20% B, 200 µl min^{-1}; 13 min, 80% B, 200 µl min^{-1}; 17 min, 100% B, 200 µl min^{-1}; 17.5 min, 100% B, 300 µl min^{-1}; 20 min, 100% B, 300 µl min^{-1}; 20.5 min, 0% B, 300 µl min^{-1}; 24 min, 0% B, 300 µl min^{-1}; 25 min, 0% B, 200 µl min^{-1}. Other liquid chromatography parameters, common to both methods, were column temperature 25 °C, autosampler temperature 5 °C, and injection volume 10 µl. Data analysis with MAVEN software and natural isotope correction was performed as previously described[40]. Volcano plot and principle component analysis of metabolomics data were generated using Metaboanalyst[47].

Acetate measurement

Acetate was derivatized and measured by LC–MS. The derivatization mixture was 12 mM EDC, 15 mM 3-nitrophenylhydrazine and pyridine (2% v/v) in methanol. The reaction was stopped with quenching reagent consisting of 0.5 mM β-mercaptoethanol in water. Serum (5 µl) was mixed with derivatizing reagent (100 µl) and incubated for 1 h at 4 °C. Then, the samples were centrifuged at 16,000*g* for 10 min at 4 °C, and 20 µl of supernatant was mixed with 200 µl of the quenching reagent. After centrifugation at 16,000*g* for 10 min at 4 °C, supernatants were collected for LC–MS analysis. A quadrupole-time-of-flight mass spectrometer (Agilent 6550 iFunnel Q-TOF) operating in negative ion mode was coupled to C18 chromatography via electrospray ionization and used to scan from *m/z* 100 to 300 at 1 Hz and 15,000 resolution. Liquid chromatography separation was on an Acquity UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 5 µm particle size, 130 Å pore size; Waters) using a gradient of solvent A (water) and solvent B (methanol). Flow rate was 200µl min^{-1}. The liquid chromatography gradient was: 0 min, 10% B; 1 min, 10% B; 5 min, 30% B; 7 min, 100% B; 11 min, 100% B; 11.5 min, 10% B; 14 min, 10% B. Autosampler temperature was 5 °C, column temperature was 60 °C, and injection volume was 10 µl. Ion masses for derivatized acetate were 194.

Lipidomics

Lipidomics was performed as previously described[48], with some modifications on an extraction step. In brief, liver powder (10 mg) was dissolved in 100 µl of isopropanol. After centrifugation at 14,000*g* at 4 °C for 10 min, supernatant was transferred to a glass mass spectrometry vial and injected into a 1290 Infinity UHPLC system coupled to the Q-TOF mass spectrometer (the same instrument as used for acetate measurement). To cover both the positive charged and negative charged species, each sample was analysed twice using the same liquid chromatography gradient but with different mass spectrometer ionization modes. The liquid chromatographyseparation was performed on an Agilent Poroshell 120 EC-C18 column (150 × 2.1 mm, 2.7 µm particle size) with a flow rate of 150 µl min^{-1}. Solvent A was 1 mM ammonium acetate plus 0.2% acetic acid in water/methanol (90:10). Solvent B was 1 mM ammonium acetate plus 0.2% acetic acid in methanol/2-propanol (2:98). The solvent gradient in volume ratios was as follows: 0–2 min, 25% B; 2–4 min, 25 to 65% B; 4–16 min, 65 to 100% B; 16–20 min, 100% B; 20–21 min, 100 to 25% B; 21–27 min, 25% B. Principle component analysis was generated using Metaboanalyst[47] (https://www.metaboanalyst.ca) and a heat map of lipidomics data was generated using Morpheus (https://software.broadinstitute.org/morpheus).

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

All data generated or analysed during this study are included in this published article (and its Supplementary Information files). Source Data for Figs. 1–4 and Extended Data Figs. 1, 4–9 are provided with the paper.

37. Postic, C. et al. Dual roles for glucokinase in glucose homeostasis as determined by liver and pancreatic beta cell-specific gene knock-outs using Cre recombinase. *J. Biol. Chem.* **274**, 305–315 (1999).
38. Nadkarni, M. A., Martin, F. E., Jacques, N. A. & Hunter, N. Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. *Microbiology* **148**, 257–266 (2002).
39. Guan, D. et al. Diet-induced circadian enhancer remodeling synchronizes opposing hepatic lipid metabolic processes. *Cell* **174**, 831–842.e12 (2018).
40. Su, X., Lu, W. & Rabinowitz, J. D. Metabolite Spectral Accuracy on Orbitraps. *Anal. Chem.* **89**, 5940–5948 (2017).
41. Titchenell, P. M., Chu, Q., Monks, B. R. & Birnbaum, M. J. Hepatic insulin signalling is dispensable for suppression of glucose output by insulin in vivo. *Nat. Commun.* **6**, 7078 (2015).
42. Iroz, A. et al. A specific ChREBP and PPARα cross-talk is required for the glucose-mediated FGF21 response. *Cell Rep.* **21**, 403–416 (2017).
43. Frey, A. J. et al. LC-quadrupole/Orbitrap high-resolution mass spectrometry enables stable isotope-resolved simultaneous quantification and ^{13}C-isotopic labeling of acyl-coenzyme A thioesters. *Anal. Bioanal. Chem.* **408**, 3651–3658 (2016).
44. Trefely, S., Ashwell, P. & Snyder, N. W. FluxFix: automatic isotopologue normalization for metabolic tracer analysis. *BMC Bioinformatics* **17**, 485 (2016).
45. Lee, J. V. et al. Acetyl-CoA promotes glioblastoma cell adhesion and migration through Ca^{2+}-NFAT signaling. *Genes Dev.* **32**, 497–511 (2018).
46. Lu, W. et al. Metabolomic analysis via reversed-phase ion-pairing liquid chromatography coupled to a stand alone orbitrap mass spectrometer. *Anal. Chem.* **82**, 3212–3221 (2018).
47. Chong, J. et al. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. *Nucleic Acids Res.* **46** (W1), W486–W494 (2018).
48. Neinast, M. D. et al. Quantitative analysis of the whole-body metabolic fate of branched-chain amino acids. *Cell Metab.* **29**, 417–429.e4 (2018).

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Author contributions The project was conceptualized and designed by S.Z., C.J. and K.E.W. K.E.W. and J.D.R. guided the study. S.Z. generated LAKO mice and performed most of the mouse experiments, with help from J.L., S.F., A.C. and K.D.M. C.J. performed mouse experiments and most of the LC–MS analyses with help from X.Z. for LC–MS analysis of short-chain fatty acid species. S.T. and N.W.S. performed LC–MS analysis of acyl-CoA species. K.U. isolated and performed experiments on primary hepatocytes. J.L., M.G. and L.I. performed experiments. P.M.T., Z.T.S. and T.P.G. provided guidance on study design. S.Z. prepared figures with input from C.J., J.D.R. and K.E.W. S.Z., C.J. and K.E.W. wrote and edited the manuscript, with input from J.D.R. All authors read and provided feedback on manuscript and figures.

Competing interests J.D.R. is a consultant to Pfizer and to Colorado Research Partners. All other authors declare no conflicts of interest.

Additional information
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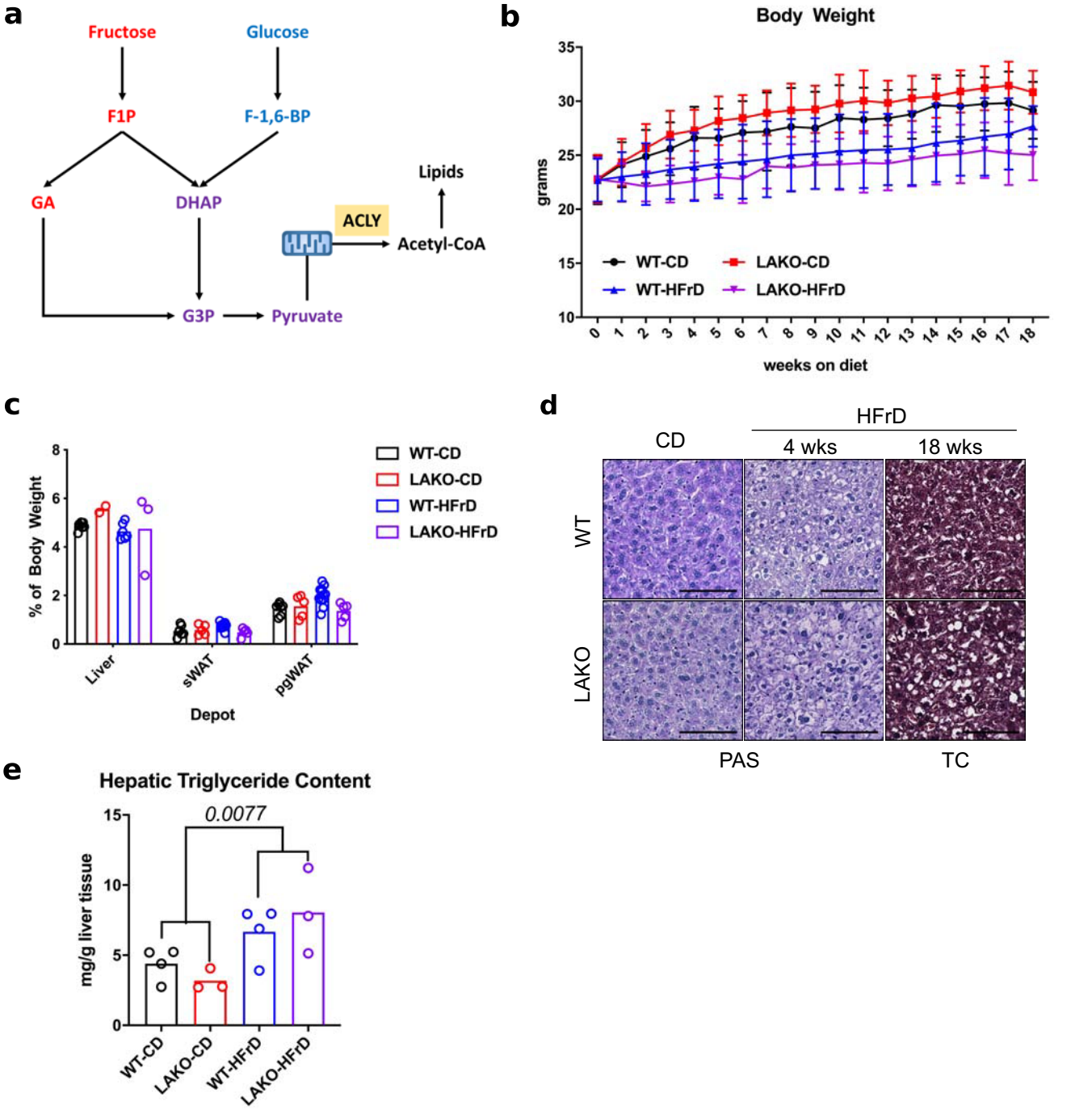
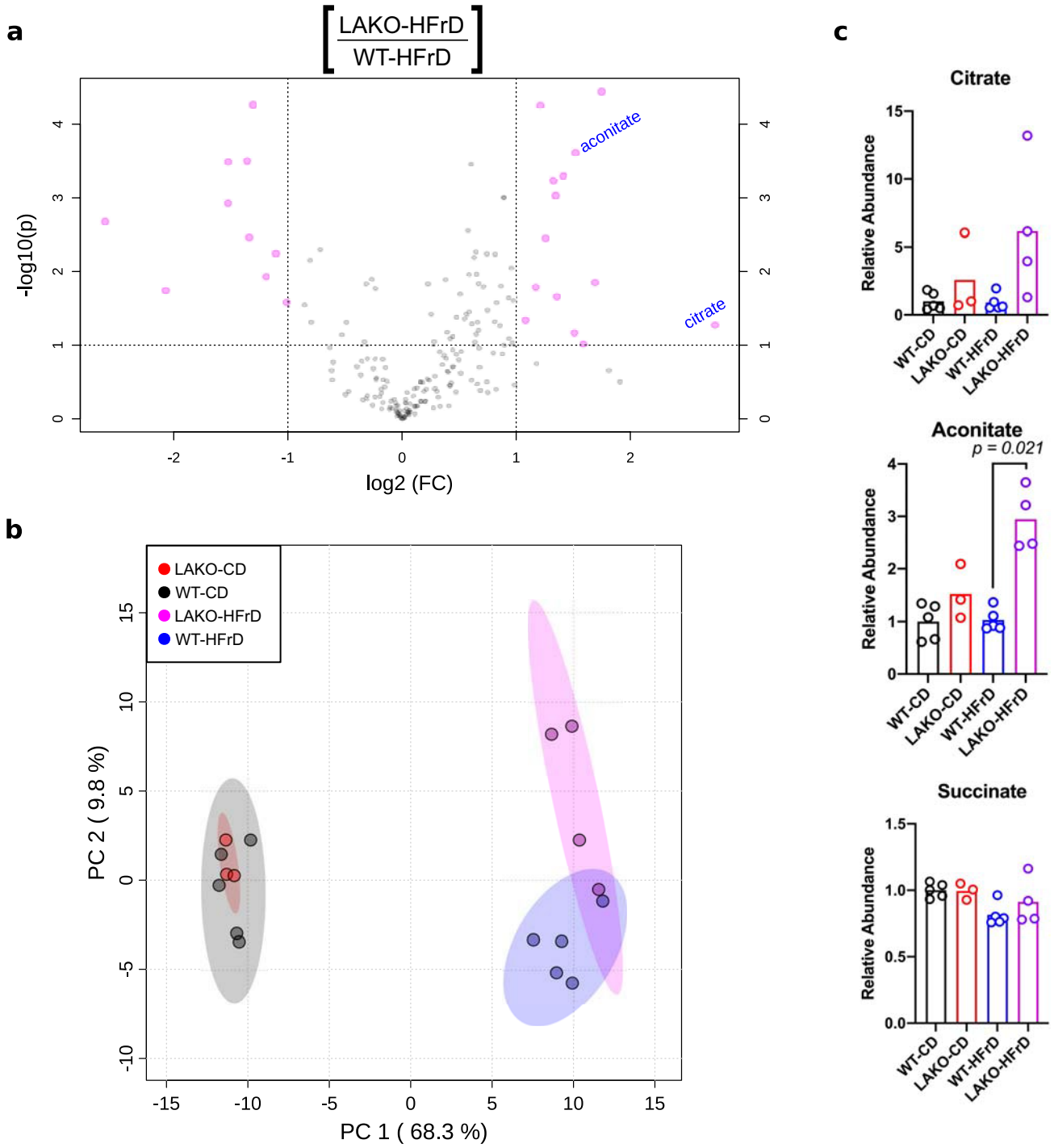
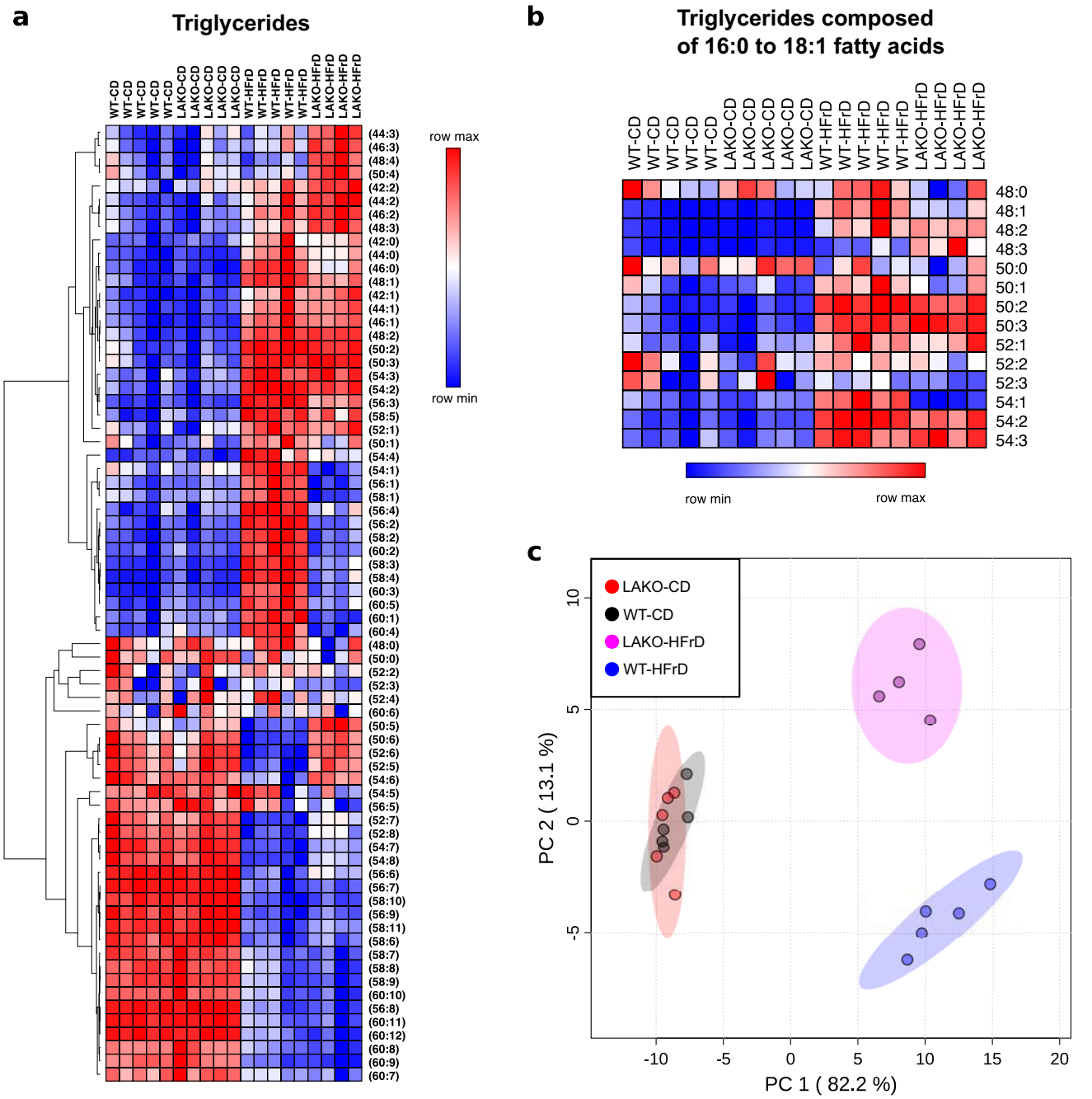


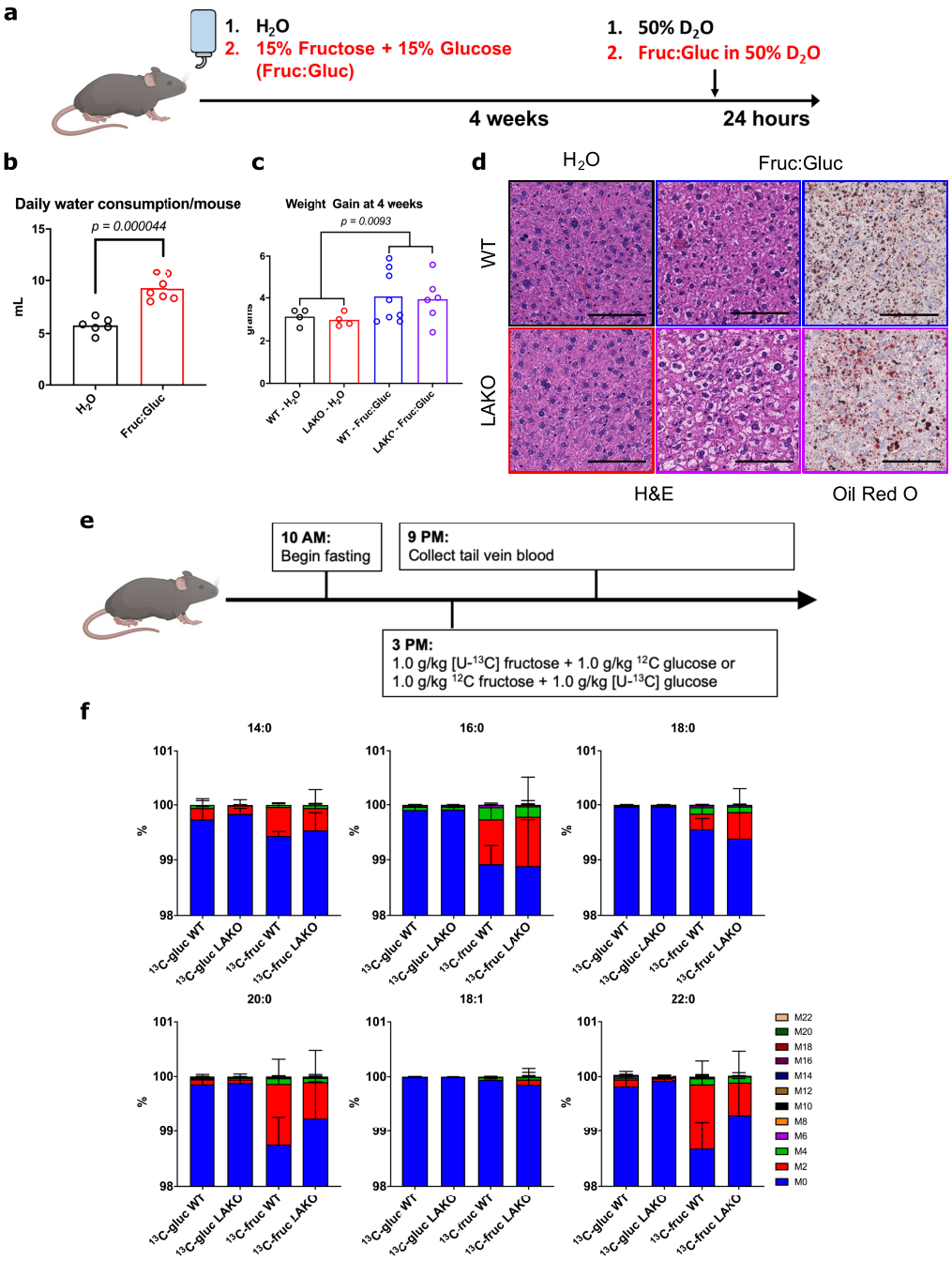
Extended Data Fig. 1 | Hepatic ACLY deficiency minimally affects the response to dietary fructose. a, Schematic of fructolysis and glycolysis feeding into de novo lipogenesis. **b**, Body weights of wild-type and LAKO mice fed a chow diet (CD) or high-fructose diet (HFrD) for 18 weeks ($n = 13$ WT-CD; $n = 5$ LAKO-CD; $n = 14$ WT-HFrD; and $n = 5$ LAKO-HFrD mice). Data are mean ± s.d. **c**, Weights of liver, posterior subcutaneous white adipose tissue (sWAT) and perigonadal adipose tissue (pgWAT) in wild-type and LAKO mice on a chow diet or HFrD for 18 weeks (liver/sWAT/pgWAT: $n = 7/7/7$ WT-CD; $n = 2/5/5$ LAKO-CD; $n = 6/12/12$ WT-HFrD; and $n = 3/5/5$ LAKO-HFrD). **d**, Representative histology images of Periodic acid–Schiff (PAS) stain for glycogen and trichrome (TC) for fibrosis in livers from wild-type or LAKO mice on a HFrD. Scale bars, 100 µm. Images are representative of two mice per group in one experiment. **e**, Triglyceride content in wild-type or LAKO mice on a chow diet or HFrD for 18 weeks ($n = 4$ WT-CD; $n = 3$ LAKO-CD; $n = 3$ WT-HFrD; $n = 4$ LAKO-HFrD). *P* values determined by Welch's *t*-test. Data in **c** and **e** denote mean values.



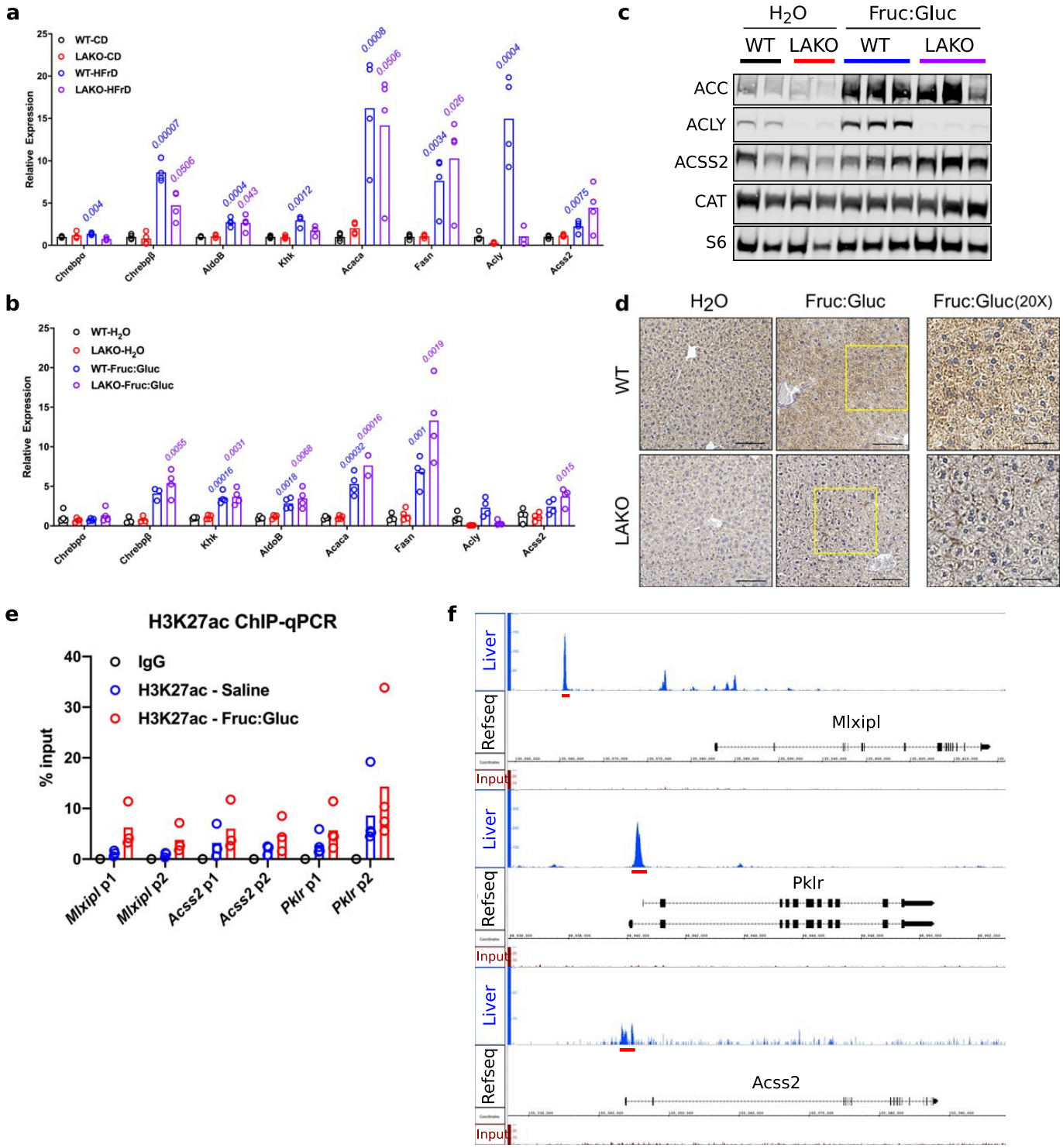
Extended Data Fig. 2 | Hepatic ACLY deficiency results in modest metabolic alterations on a high-fructose diet. a, Volcano plot of hepatic metabolites in wild-type or LAKO mice on a chow (CD) or high-fructose diet (HFrD) for 4 weeks. Pink dots indicate significant hits as determined by a fold-change (FC) threshold of 2.0, and *P*-value threshold of 0.1, assuming equal variance. **b**, Principle component (PC) analysis of log-transformed data in Supplementary Table 1. Each dot represents a unique sample; coloured shading denotes 95% confidence intervals. **c**, Relative abundance of metabolites, normalized to the wild-type chow-diet-fed (WT-CD) group. *P* values determined by Welch's *t*-test ($n = 5$ WT-CD; $n = 3$ LAKO-CD; $n = 5$ WT-HFrD; and $n = 4$ LAKO-HFrD mice). Data are mean values.



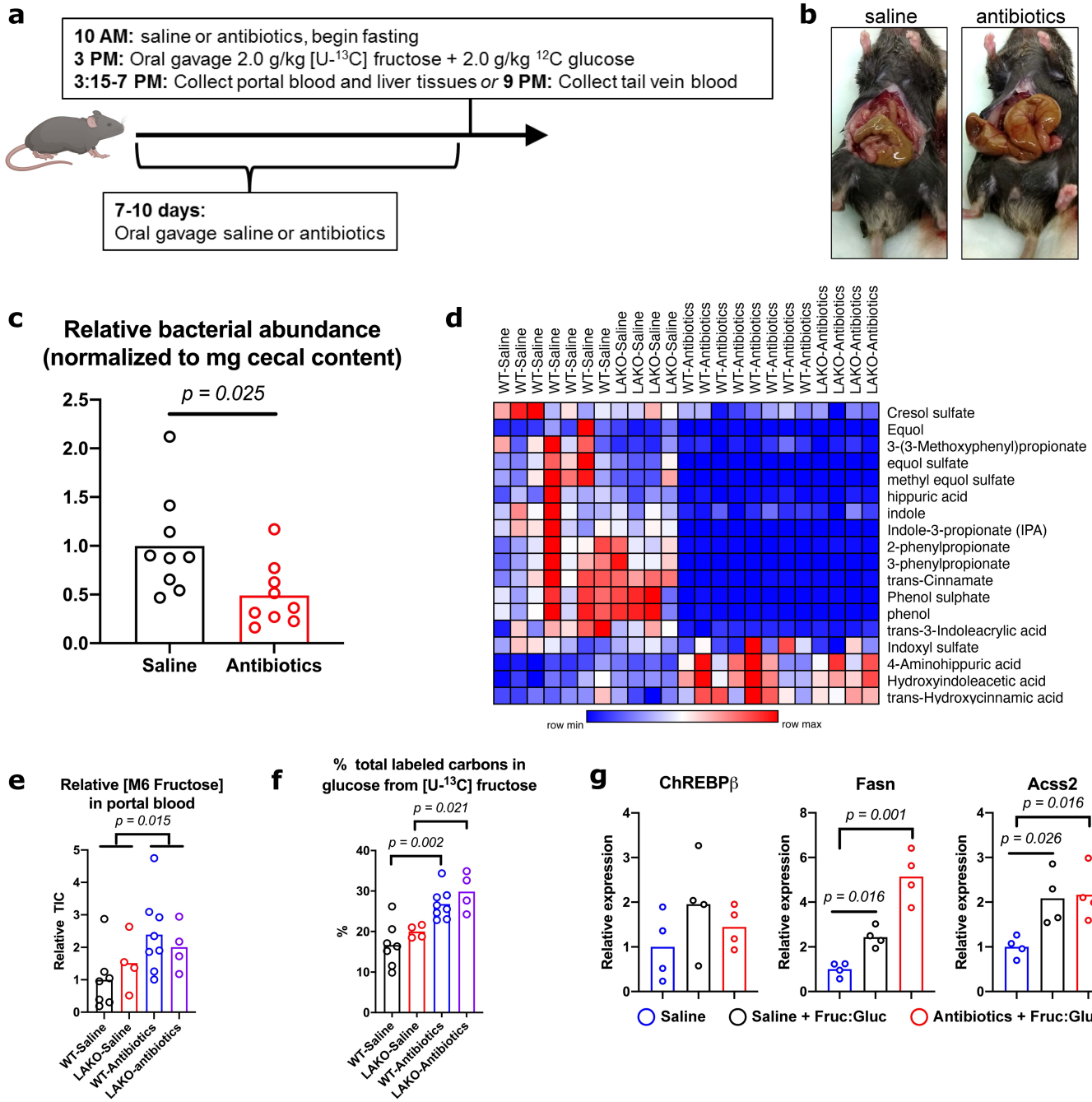
Extended Data Fig. 3 | A high-fructose diet alters hepatic lipid metabolism. **a**, Hierarchical clustering of relative hepatic triglyceride abundance in wild-type or LAKO mice on a chow diet (CD) or high fructose diet (HFrD) for 4 weeks. Clustering performed using 1 − Pearson's correlation and average linkage. **b**, Relative abundance of hepatic triglycerides composed of 16:0 to 18:1 fatty acids; a subset of the data in **a**. **c**, Principle component analysis of log-transformed data in Supplementary Table 2. Each dot represents a unique sample; coloured shading denotes 95% confidence intervals.



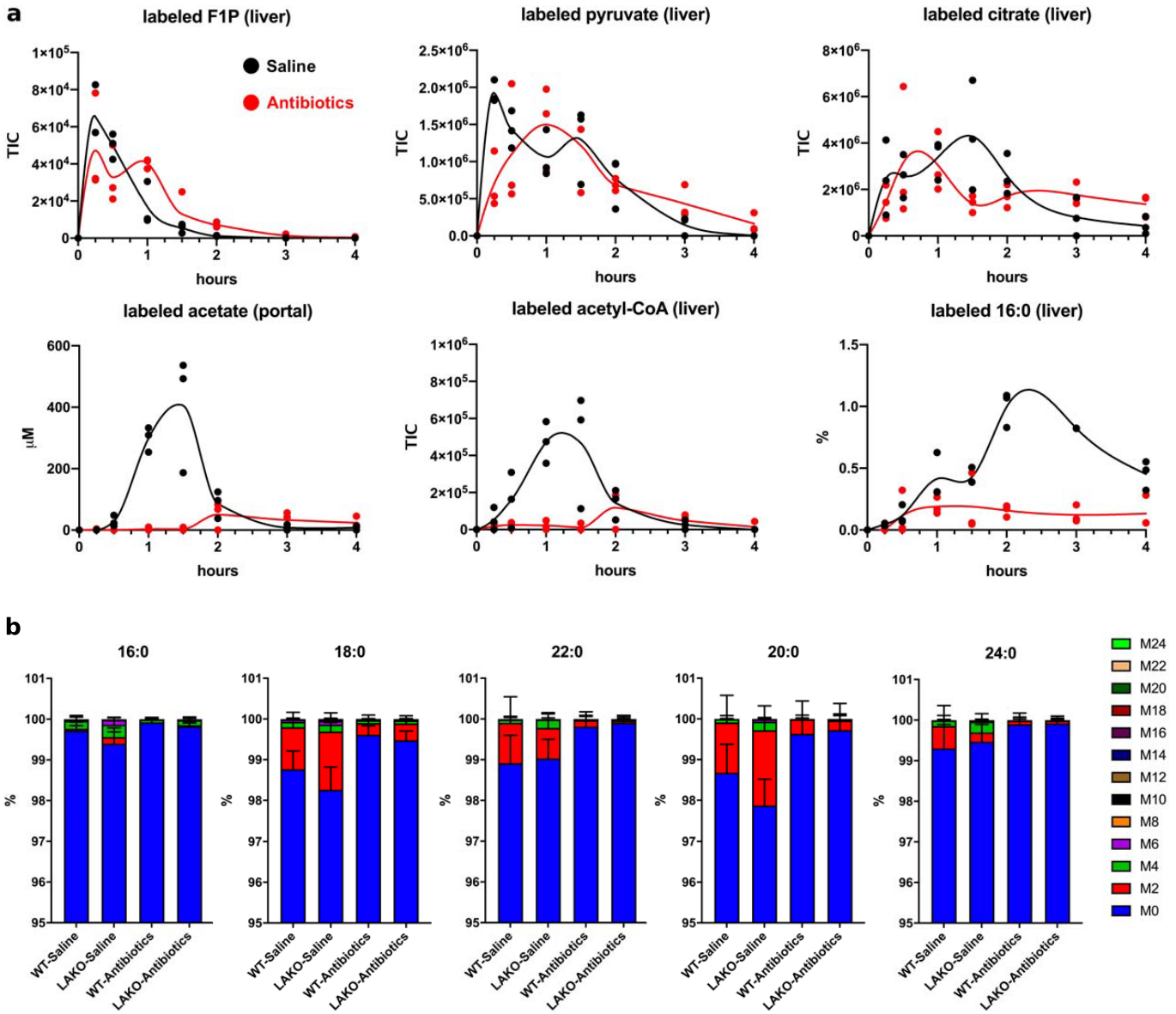
Extended Data Fig. 4 | Fructose induces steatosis and contributes substantially to newly synthesized fatty acids in the liver independently of ACLY. a, Schematic of experimental design of the drinking water study. **b**, Daily consumption of unsweetened (H_2O) or 15% fructose and 15% glucose sweetened (Fruc:Gluc) water per mouse. Each dot represents a repeat measurement, and mean values are shown ($n = 6$ H_2O, $n = 7$ Fruc:Gluc). *P* values determined by Welch's *t*-test. **c**, Weight gain of wild-type or LAKO mice given water or fructose:glucose for 4 weeks ($n = 4$ WT-H_2O, LAKO-H_2O; $n = 8$ WT-Fruc:Gluc; and $n = 6$ LAKO-Fruc:Gluc mice). *P* values comparing all H_2O versus fructose:glucose mice determined by Welch's *t*-test. **d**, Representative H&E and Oil Red O histological stains of livers from mice in **c**. Scale bars, 100 µm. **e**, Experimental design for data in Fig. 1c. [U-^{13}C] denotes uniformly labelled ^{13}C. **f**, Isotopologue distribution of labelled saponified fatty acids in serum shown in Fig. 1c. Data are mean ± s.d.



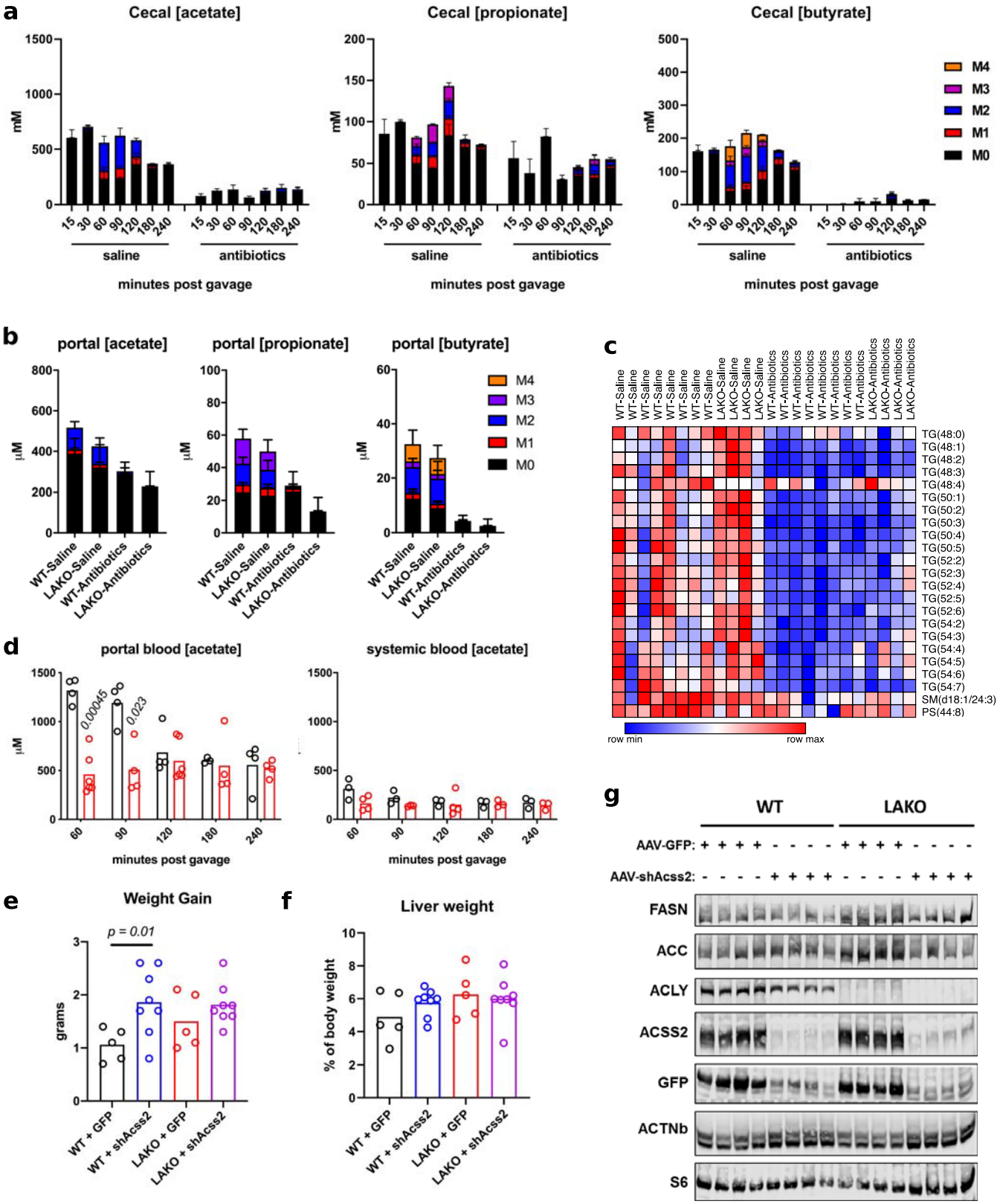
Extended Data Fig. 5 | Fructose signals the use of acetate for de novo lipogenesis. a, mRNA expression of *ChREBP* and its target genes in livers of wild-type or LAKO mice fed a chow or high-fructose diet ($n = 4$ mice per group). *P* values for WT-CD versus WT-HFrD (blue text) and for LAKO-CD versus LAKO-HFrD (purple text) determined by two-sided *t*-tests with Holm–Sidak method for multiple comparisons. **b**, mRNA expression of lipogenic genes in livers of wild-type or LAKO mice given H_2O or fructose:glucose water for 4 weeks ($n = 4$ mice per group). *P* values for WT-H_2O versus WT-Fruc:Gluc, WT-H_2O versus WT-Fruc:Gluc (blue font) and LAKO-H_2O versus LAKO-Fruc:Gluc (purple font) were determined by two-sided *t*-test with Holm–Sidak method for multiple comparisons. **c**, Western blots of lipogenic enzymes in liver lysates of wild-type or LAKO mice given H_2O or fructose:glucose water for 4 weeks. Each lane represents an individual mouse. **d**, Immunohistochemistry staining analysis of ACLY in livers from wild-type or LAKO mice given H_2O or fructose:glucose water for 4 weeks. Yellow boxes mark the approximate location of the ×20 panels. Scale bars, 100 μm and 50 μm (for ×20). **e**, H3K27ac ChIP–quantitative PCR (qPCR) analysis of livers from wild-type mice provided either water for 24 h followed by an oral gavage of saline, or fructose:glucose water for 24 h followed by an oral gavage of 2.0 g kg^{-1} glucose and 2.0 g kg^{-1} fructose ($n = 3$ *Mlxipl*, *Acss2*; $n = 4$ *Pklr*). Livers were obtained 90 min after gavage. 'p1' and 'p2' are two different primer sets. **f**, ChIP–seq tracks of *Mlxipl*, *Pklr* and *Acss2* genomic loci[16]. Red bars indicate genomic regions used to design ChIP–qPCR primers. Data in **a**, **b**, and **e** denote mean values.



Extended Data Fig. 6 | Depletion of microbiota blocks substrate contribution, but not signalling component, of de novo lipogenesis after fructose consumption. a, Experimental set-up for antibiotic depletion of the microbiome followed by [^{13}C]fructose tracing into DNL. [U-^{13}C] denotes uniformly labelled ^{13}C. **b**, Representative images of caecums from a mouse treated with saline or antibiotics. **c**, Relative abundance of bacteria in caecal contents from mice treated with saline ($n = 9$) or antibiotics ($n = 9$), as determined by *16S* RT–qPCR to a reference standard of *Escherichia coli* DNA. *P* value determined using Welch's *t*-test. **d**, Heat map of microbial metabolite abundance in the portal blood, collected 1 h after gavage. **e**, **f**, Relative abundance of [^{13}C]fructose (**e**) and percentage of total labelled carbons in glucose (**f**) in portal blood from wild-type or LAKO mice treated with saline or antibiotics, collected 1 h after gavage ($n = 7$ WT-saline, WT-antibiotics; and $n = 4$ LAKO-saline, LAKO-antibiotics). *P* values determined by Welch's *t*-test. **g**, mRNA expression of *ChREBPb*, *Acss2* and *Fasn* in liver collected 1 h after gavage ($n = 4$ mice per group). *P* values determined by two-sided *t*-tests with Holm–Sidak method for multiple comparisons. Data in **c** and **e**–**g** denote mean values.

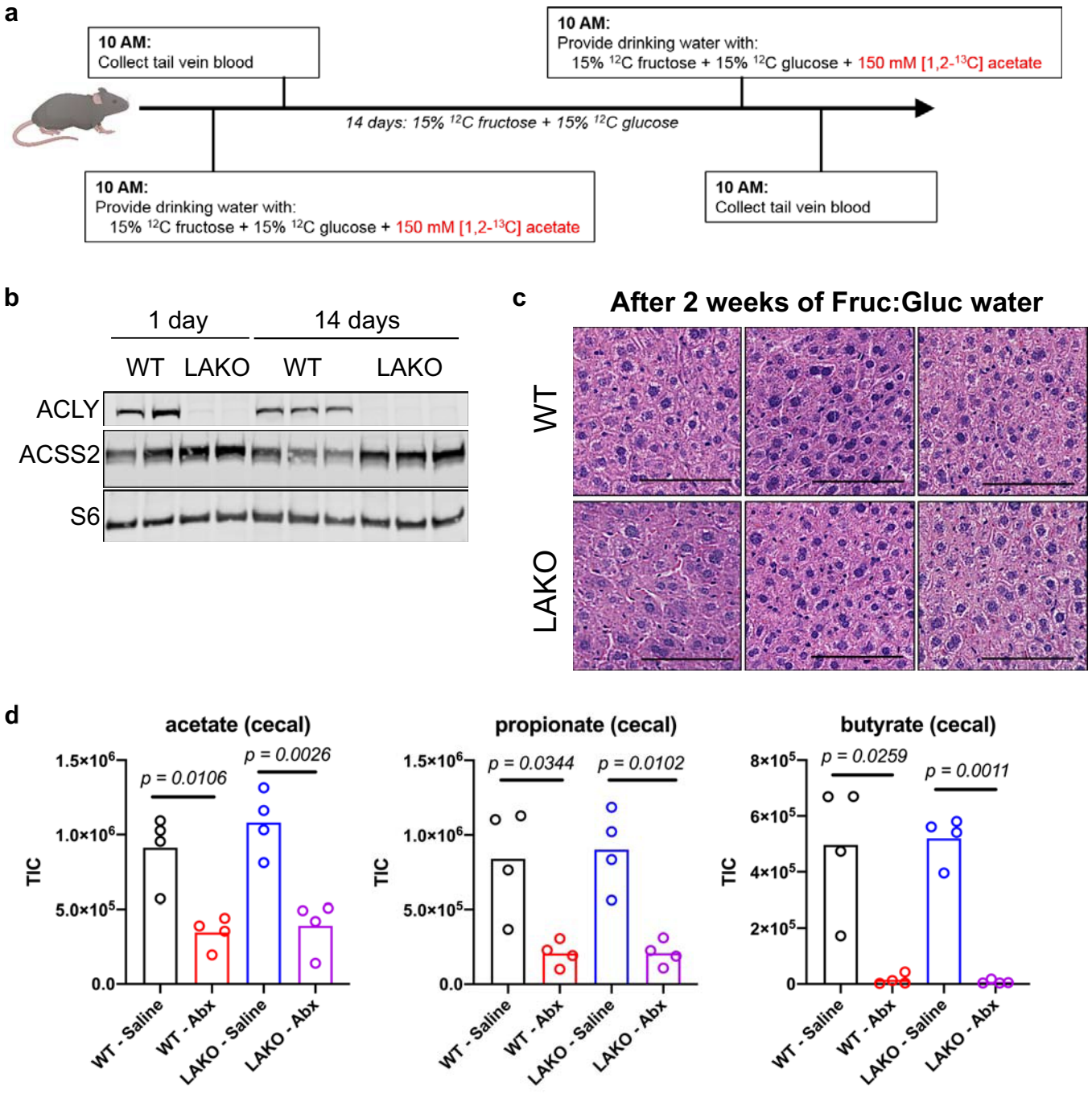


Extended Data Fig. 7 | Bolus fructose is converted into acetate in a microbiota-dependent manner. a, TIC of labelled F1P, pyruvate, citrate and acetyl-CoA in liver, concentration of labelled acetate in portal blood, and percentage of labelled carbons in hepatic saponified fatty acids from wild-type mice treated with saline or antibiotics, and gavaged with 2.0 g kg^{-1} [^{13}C]fructose plus 2.0 g kg^{-1} unlabelled glucose (n = 3 mice per time point). Data for saline-treated mice are also shown in Fig. 2d. **b**, Isotopologue distribution of saponified fatty acids in serum from wild-type or LAKO mice fed and treated as in Fig. 3b, and collected 6 h after gavage (n = 8 WT-saline, WT-antibiotics; n = 4 LAKO-saline, LAKO-antibiotics). Data are mean ± s.d.

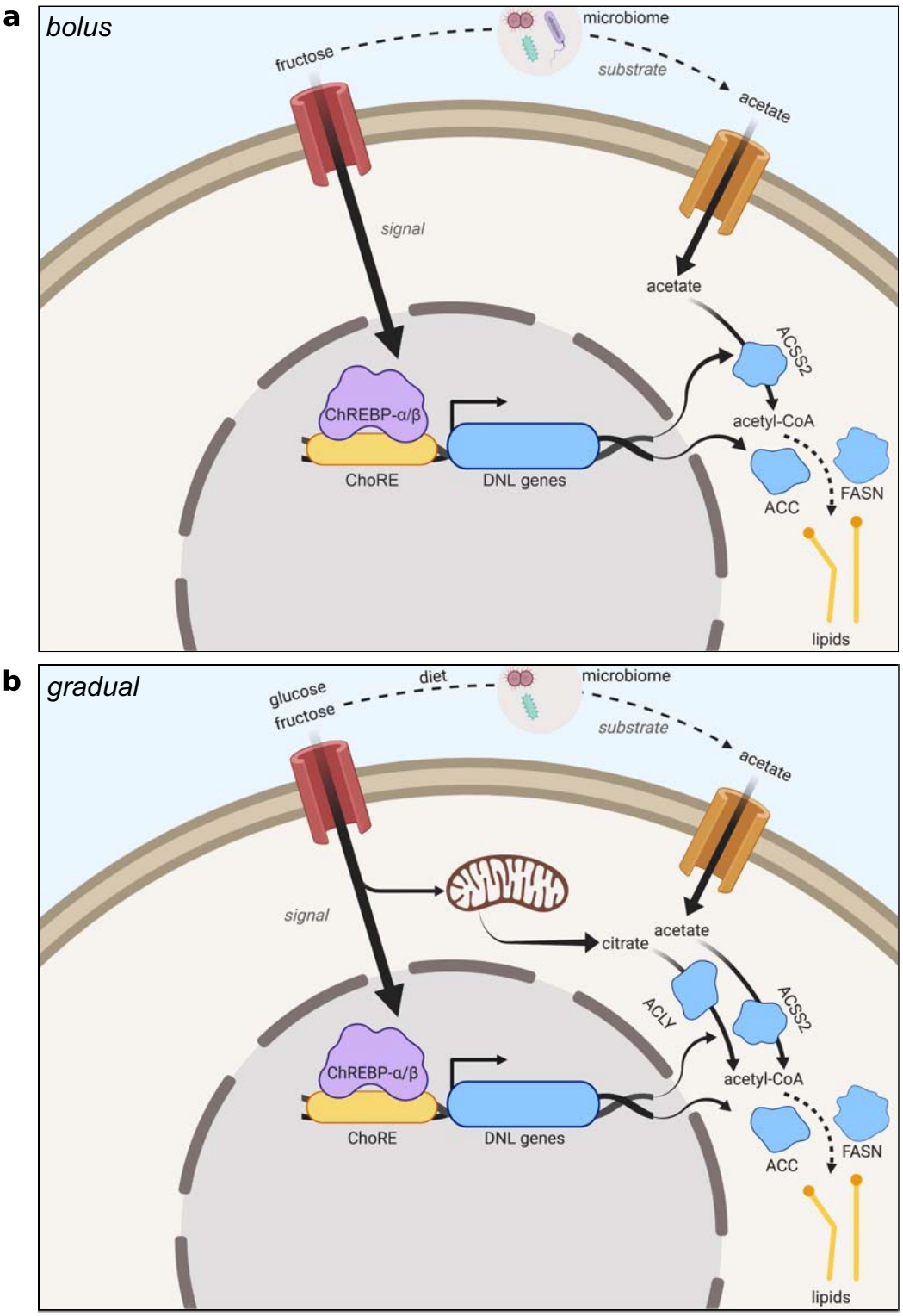


Extended Data Fig. 8 | See next page for caption.

Extended Data Fig. 8 | Bolus fructose-dependent DNL requires microbial acetate and hepatic ACSS2. a–**d**, Mice were gavaged with 2.0 g kg^{-1} [^{13}C]fructose and 2.0 g kg^{-1} unlabelled glucose. **a**, Concentrations of labelled acetate, propionate and butyrate in caecal contents from wild-type mice treated with saline or antibiotics (n = 3 mice per time point, except for saline-180 n = 2 mice). **b**, Concentrations of labelled acetate, propionate and butyrate in portal blood from wild-type mice treated with saline or antibiotics (n = 8 WT-saline, WT-antibiotics; and n = 4 LAKO-saline, LAKO-antibiotics), collected 1 h after gavage. **c**, Heat map of hepatic triglyceride abundance in livers of mice treated with saline or antibiotics. **d**, Concentrations of acetate in portal and systemic blood after gavage. Each data point represents an individual mouse. P value determined by two-sided t-tests with Holm–Sidak method for multiple comparisons. **e**, Weight gain in wild-type and LAKO mice 1 week after tail-vein injection of AAV8-GFP or AAV8-shAcss2. P value determined by Welch's t-test. **f**, Liver weight of wild-type and LAKO mice as a percentage of body weight 1 week after tail-vein injection of AAV8-GFP or AAV8-shAcss2. **g**, Western blots of lipogenic enzymes in liver lysates from wild-type and LAKO mice 1 week after tail-vein injection of AAV8-GFP or AAV8-shAcss2. S6 was used as a loading control.



Extended Data Fig. 9 | Gradual fructose consumption promotes greater acetate usage in LAKO mice. a, Experimental set-up for tracing of [1,2-^{13}C]acetate into DNL before and after gradual administration of fructose. [1,2-^{13}C] denotes ^{13}C labelling of 1 and 2 position carbons in acetate. **b**, Western blots of lipogenic enzymes in liver lysates from wild-type and LAKO mice after being given fructose:glucose water for 1 or 14 days. **c**, Representative H&E stains of livers from wild-type and LAKO mice provided fructose:glucose water for 2 weeks. Scale bars, 100 µm. **d**, Relative abundance of acetate, propionate and butyrate in the caecal contents of wild-type and LAKO mice treated with saline or antibiotics for 1 week ($n = 4$ mice per group). *P* values determined by Welch's *t*-test.



Extended Data Fig. 10 | Fructose provides signal and substrate to promote hepatic DNL. a, Proposed model of bolus fructose-induced hepatic DNL. Fructose catabolism in hepatocytes acts as a signal to induce DNL genes including *Acss2*, whereas fructose metabolism by the gut microbiota provides acetate as a substrate to feed DNL, which is mediated by ACSS2. **b**, Proposed model of gradual fructose-induced hepatic DNL. Similar to the bolus model, fructose catabolism in hepatocytes acts as a signal to induce DNL genes. Catabolism of hepatic fructose and glucose (made from fructose by the small intestine) provides citrate as a substrate to feed DNL, which is mediated by ACLY. Metabolism of fibres and other dietary components by the gut microbiota provides acetate as a substrate to feed DNL, after its conversion to acetyl-CoA by hepatic ACSS2. Image created with BioRender.com.

nature research

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☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Data collection for metabolomics was done using Xcaliber Qual Browser (Thermo Fisher) and TraceFinder (Thermo Fisher)
Data analysis	Data analysis was performed using MAVEN, FluxFix, and GraphPad Prism versions 7 and 8.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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All data generated or analysed during this study are included in this published article (and its supplementary information files).

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample sizes were determined in accordance with the literature and based on previous experience in our group.
Data exclusions	No data were excluded from the analyses in this study.
Replication	Experimental findings were verified by using multiple mice in each study as replicates. Replications were successful.
Randomization	Mice of different genotypes were randomly assigned to treatment groups throughout this study.
Blinding	Sample preparation and data acquisition for metabolomics were performed in a blinded manner.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	ACLY (Proteintech, Catalogue #: 15421-1-AP, Lot: 00040639), ACSS2 (Cell Signaling Technology, Catalogue #: 3658, Clone: D19C6, Lot: 2), ACC (Cell Signaling Technology, Catalogue #: 3676, Clone: C83B10, Lot: 9), FASN (Cell Signaling Technology, Catalogue #: 3189, Lot: 2), CHREBP (Novus Biologicals, Catalogue #: NB400-135, Lot: H-1), IgG (Cell Signaling Technology, Catalogue #: 2729, Lot: 8), CAT (Cell Signaling Technology, Catalogue #: 14097, Clone: D5N7V, Lot: 1), S6 (Cell Signaling Technology, Catalogue #: 2217, Lot: 7), Acetyl-H3K27 (Abcam, Catalogue #: ab4729, Lot: GR3216173-1)
Validation	All antibodies were used in accordance to the manufacturer guidelines and confirmed to have previously been documented in the literature through CiteAb (www.citeab.com).

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Mice used in this study were on a C57bl/6j background, all males, and between the ages of 8-12 weeks for acute experiments. Long-term diet studies were started when mice were around 6-8 weeks of age.
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	All animal protocols in this study were approved by the Institutional Animal Care and Use Committees (IACUC) at the University of Pennsylvania and Princeton University.

Note that full information on the approval of the study protocol must also be provided in the manuscript.