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Carbon fluxes and differential glucose usages in *Francisella* strains, *Bacillus subtilis* and thermogenic brown adipocytes

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古之欲明明德於天下者、先治其國。欲治其國者先齊其家。欲齊其家者先脩其身。欲脩其身者先正其心。欲正奇心者先誠其意。欲誠其意者先致其知。致知在格物。物格而后知至。知至而后意誠。意誠而后心正。心正而后身脩。身脩而后家齊。家齊而后國治。國治而后天下平。

大學

The ancients who wanted to manifest their bright virtue to all in the world first governed well their own states. Wanting to govern well their states, they first harmonized their own clans. Wanting to harmonize their own clan, they first cultivated themselves. Wanting to cultivate themselves, they first corrected their minds. Wanting to correct their minds, they first made their wills sincere. Wanting to make their wills sincere, they first extended their knowledge. Extension of knowledge consists of the investigation of things. When things are investigated, knowledge is extended. When knowledge is extended, the will becomes sincere. When the will is sincere, the mind is correct. When the mind is correct, the self is cultivated. When the self is cultivated, the clan is harmonized. When the clan is harmonized, the country is well governed. When the country is well governed, there will be peace throughout the land.

The Great Learnin

List of publications

Chen, F.; Rydzewski, K.; Kutzner, E.; Häuslein, I.; Schunder, E.; Wang, X.; Meighen-Berger, K.; Grunow, R.; Eisenreich, W.; Heuner, K., Differential Substrate Usage and Metabolic Fluxes in *Francisella tularensis* Subspecies *holarctica* and *Francisella novicida*. Frontiers in cellular and infection microbiology 2017, 7, 275.

Further publications

Bacher, A.; Chen, F.; Eisenreich, W., Decoding biosynthetic pathways in plants by pulse-chase strategies using $^{13}CO_2$ as a universal tracer. Metabolites 2016, 6 (3), 21.

Häuslein, I.; Cantet, F.; Reschke, S.; Chen, F.; Bonazzi, M.; Eisenreich, W., Multiple substrate usage of *Coxiella burnetii* to feed a bipartite-type metabolic network. Frontiers in cellular and infection microbiology 2017, 7, 285.

Summary

Using labelling experiments and isotopologue profiling, metabolic pathways, substrate usages and fluxes in different systems (pathogenic bacteria, bacteria producing biofilms and brown fat cells from mice) could be analysed. Labelled glucose, glutamate, glycerol and serine were supplied during the cultivation of the bacteria or cells, which were incorporated and utilized in downstream metabolism. Using physical and chemical methods, downstream products could be isolated and analysed by gas chromatography/mass spectrometry (GC/MS). By comparison of these patterns, the pathways involved in the metabolism of the supplemented tracers could be reconstructed for the various bacteria and eukaryotic cells in considerable details. In this work, we especially studied differential glucose usages and carbon fluxes in *Francisella* strains, *Bacillus subtilis* and immortal brown adipose tissue.

Francisella tularensis is an intracellular pathogenic bacterium which can infect many animals causing tularemia. The metabolic fluxes and pathways of *F. tularensis* subsp. *holarctica*, the highly pathogenic wild type strain, and *F. novicida*, the human avirulent but mice virulent strain, were studied in order to find out the relationship between virulence and metabolism. We performed ^{13}C-labeling experiments with *F. tularensis* subsp. *holarctica* wild type (beaver isolate), *F. tularensis* subsp. *holarctica* strain LVS, or *F. novicida* strain U112 in complex media containing either [U-$^{13}C_6$]glucose, [1,2-$^{13}C_2$]glucose, [U-$^{13}C_3$]serine, or [U-$^{13}C_3$]glycerol. On the basis of GC/MS analysis, the isotopologue profiling of amino acids, polysaccharide-derived glucose, free fructose, amino sugars derived from the cell wall, fatty acids, 3-hydroxybutyrate, lactate, succinate and malate could reveal uptake and metabolic usage of all tracers. The two *F. tularensis* strains, i.e. the beaver isolated *holarctica* wild type strain and the live vaccine strain LVS, showed similar labeling patterns. However, the differences between the labelling profiles of the metabolites from the *F. tularensis* strains and *F. novicida* strain were significant. In *F. tularensis* subsp. *holarctica* strains, glucose was directly used for polysaccharide and cell wall biosynthesis with higher rates, however, in the *F. novicida* strain, glucose was more efficiently used for the biosynthesis of downstream products (e.g. amino aicds, TCA intermediates) via the EMP pathway or the PPP. Catabolic turnover of glucose via gluconeogenesis was also observed. Although pyruvate transaminase is not annotated in the genomes of *F. tularensis* and *F. novicida*, Ala could still be synthesized from pyruvate. The glycerol could be efficiently uptaken by *F. novicida* serving as a gluconeogenetic substrate, however, for *F. tularensis* subsp. *holarctica* strains, glycerol usage for gluconeogenetic turnover was less. Serine was not a major substrate for both *F. tularensis* subsp. *holarctica* strains and *F. novicida* strain. It was utilized, at low rates, for the biosynthesis of downstream metabolites, e.g. TCA intermediates and fatty acids, especially in the *F. tularensis* subsp. *holarctica* strains. However, serine was almost not ultilized for gluconeogenesis under the experimental conditions. It was speculated that the different utilization of substrates could be related to

host specificity and virulence of *Francisella*.

Francisella sp. strain W12-1067 is a recently in Germany identified new environmental *Francisella* isolate, which is negative for the typical *Francisella* pathogenicity island FPI. However, in its genome an alternative putative T6SS is present. Therefore, it is still unknown whether this strain is pathogenic or not, the metabolism of *Francisella* is important to replicate in host cells and, thus, to be a successfull human pathogen. Therefore, we performed ^{13}C-labeling experiments with *Francisella* sp. strain W12-1067 in complex media again containing either [U-$^{13}C_6$]glucose, [1,2-$^{13}C_2$]glucose, [U-$^{13}C_3$]serine, or [U-$^{13}C_3$]glycerol. Moreover, *in silico* genome analysis revealed the presence of a putative myo-inositol metabolic pathway in *Francisella* sp. strain W12-1067. Therefore, we performed additional ^{2}H-labelling experiments with *Francisella* sp. strain W12-1067 in CDM medium containing myo-inositol-C-$^{2}H_6$. The data revealed that myo-inositol could be uptaken by *Francisella* sp. strain W12-1067. Via the myo-inositol/glucuronate pathway, 2-dehydro-3-deoxy-D-gluconate is formed, which converts later into pyruvate and glyceraldehyde 3-phosphate serving for the biosynthesis of glycogen, protein and fatty acids. In addition, glucose, serine and glycerol could be well utilized by *Francisella* sp. strain W12-1067, and were converted into sugars, protein derived amino acids, free amino acids and free metabolites. As the other *Francisella* strains, we found that glucose is the most efficient precursor and it could be degraded via EMP but not via the ED or PPP into glycogen, cell wall amino sugars, amino acids, and free fatty acids. Pyruvate transaminase is also not annotated in the the genome of *Francisella* sp. strain W12-1067. However, Ala could still be synthesized from pyruvate. Serine could not be ultilized for the gluconeogenesis under the experimental conditions, indicating substrate specificities in a bipartite metabolic network. Moreover, the Δmyo-inositol mutant of *Francisella* sp. strain W12-1067 leads to higher carbon flux to Ala (pyruvate) and lower flux to Tyr, indicating that the Eda enzyme in the wild-type strain may not only be involved in the metabolism of myo-inositol. The absence of Eda may lead to a higher concentration of pyruvate and glyceraldeyde 3-phosphate, since there is no carbon flux to 2-dehydro-3-deoxy-D-gluconate.

Bacterial biofilms are characterized by the formation of an extracellular matrix that consists of extracellular polysaccharides, proteins, and sometimes also DNA. This matrix encapsulates the cells in three-dimensional architectures. Notably, this protective environment also contributes to reduced activities of antibacterial drugs and, thus, to more resistances in the chemotherapy of bacterial infections. As the best studied Gram-positive bacterium, *Bacillus subtilis* serves as an excellent model to study the metabolic basis of biofilm formation. SinR is the master regulator of biofilm genes. It acts by binding to the promoter regions of the *tapA-sipW-tasA* and the *epsA-O* operons, encoding the extracellular amyloid and the enzymes for extracellular polysaccharide synthesis, respectively. The phosphodiesterase YmdB was shown to be required for the expression of biofilm genes. In this work,

we performed labelling experiments of three *B. subtilis* mutant growing under planktonic condition or on agar plate, using [U-$^{13}C_6$]glucose or [U-$^{13}C_5$]glutamate as precursors. More specifically, the undomesticated strain NCIB 3610, the Δ*ymdB* mutant of NCIB3610 strain (GP921), which is defective in biofilm formation, and the Δ*sinR* mutant of NCIB3610 strain (GP1562) were investigated. For the planktonic experiments, without a surface for the biofilm formation, it was shown that Δ*sinR* and Δ*ymdB* didn't play a significant role. However, on the agar plate, significant metabolic differences between the three mutants were found, which could relate to the biofilm (extracellular polysaccharide with amino acids chain) formation.

Brown adipose tissue (BAT) locates mainly in neck and thorax, whose amount depends on various factors, such as age, gender, environmental temperature, etc. Uncoupling protein 1 (UCP1), which was found in the inner membrane of mitochondria, plays a central role in the thermogenesis process of BAT. UCP1 can be activated by free fatty acids which derived from triacylglycerols by a lipase and can be inhibited by purine nucleotides. Till now, the function of UCP1 related to the metabolism is still unknown. Isoproterenol could trigger the release of fatty acids in BAT, which then activate UCP1. We performed labelling experiments with immortal BAT (IBAT) cells growing in DMEM medium containing [U-$^{13}C_6$]glucose. During the cultivation, isopretrenol was added to activate UCP1. After the activation, (i) glucose flux into glycogen storage was found to be increased, (ii) more lactate was produced from glucose, and (iii) carbon flux was directed into the oxidative TCA cycle probably to generate more energy.

Zusammenfassung

Mithilfe von Markierungsexperimenten und „Isotopologue Profiling“ konnten Stoffwechselwege und Flüsse in verschiedenen Systemen (pathogene Bakterien, Bakterien produzierende Biofilme und braunen Fettzellen) analysiert werden. Markierte Glucose, Glutamat, Glycerin und Serin wurden während der Kultivierung von Bakterien oder Zellen zugegeben und in die Stoffwechselprozesse eingebaut. Mit physikalischen und chemischen Methoden konnten Stoffwechselprodukte isoliert und mittels Gaschromatographie / Massenspektrometrie (GC / MS) analysiert werden. Durch Vergleich dieser Muster konnten die Stoffwechselwege ausgehend von den isotopmarkierten Tracern für die verschiedenen Bakterien und eukaryotischen Zellen in beträchtlichen Details rekonstruiert werden. In dieser Arbeit untersuchten wir insbesondere differentielle Glukoseverwertungen und Kohlenstoffflüsse in *Francisella*-Stämmen, *Bacillus subtilis* und braunem Fettgewebe.

Francisella tularensis ist ein intrazelluläres pathogenes Bakterium, das viele Tiere infizieren kann und dabei der Verursacher von Tularämie ist. Die metabolischen Flüsse und Stoffwechselwege von *F. tularensis* subsp. *holarctica*, der hochpathogene Wildtyp-Stamm, und *F. novicida*, der humane avirulente, aber virulente Stamm für Mäuse, wurden untersucht, um den Zusammenhang zwischen Virulenz und Metabolismus herauszufinden. Wir führten ^{13}C-Markierungsexperimente mit *F. tularensis* subsp. *holarctica* Wildtyp (Biberisolat), *F. tularensis* subsp. *holarctica* LVS oder *F. novicida* U112 in komplexen Medien durch, die entweder [U-$^{13}C_6$]Glucose, [1,2-$^{13}C_2$]Glucose, [U-$^{13}C_3$]Serin oder [U-$^{13}C_3$]Glycerin enthielten. GC/MS-Analysen konnten die Isotopologmuster von Aminosäuren, Polysaccharid-abgeleiteten Glucose, freier Fructose, Aminozuckern aus der Zellwand, Fettsäuren, 3-Hydroxybutyrat, Lactat, Succinat und Malat aufzeigen uns somit die jeweiligen Raten der Aufnahme und metabolische Nutzung belegen. Die beiden *F. tularensis*-Stämme, Biber-isolierte *holarctica* Wildtyp und LVS, zeigten ähnliche Markierungsmuster, jedoch waren die Unterschiede zwischen den Markierungsprofilen der Metaboliten von *F. tularensis*-Stämmen und *F. novicida*-Stamm signifikant. In *F. tularensis* subsp. *holarctica* wurde Glucose direkt für Polysaccharid und Zellwand Biosynthese mit höheren Raten verwendet, jedoch im *F. novicida* Stamm wurde Glukose für die Biosynthese von Downstream-Produkten (z.B. Aminosäuren und TCA Zwischenprodukten) über den EMP oder PPP mit höheren Raten verwendet. Ein katabolischer Turnover von Glukose durch Gluconeogenese wurde ebenfalls beobachtet. Obwohl die Pyruvat-Transaminase in den Genomen von *F. tularensis* und *F. novicida* nicht annotiert ist, konnte Ala immer noch aus Pyruvat synthetisiert werden. Das Glycerol konnte von *F. novicida* effizient aufgenommen werden, diente jedoch als ein glukoneogenetisches Substrat für die *F. tularensis* subsp. *holarctica* Stämme. Glycerin diente für den gluconeogenetischen Umsatz weniger. Serin war kein Hauptsubstrat für die *F. tularensis* subsp. *holarctica*-Stämme und den *F. novicida*-Stamm, wurde aber mit niedrigen Raten für die Biosynthese von Downstream-Metaboliten,

z.B. TCA-Zwischenprodukte und Fettsäuren, insbesondere in der *F. tularensis* subsp. *holarctica*-Stämme, verwendet. Serin wurde jedoch unter den experimentellen Bedingungen für die Gluconeogenese fast nicht benutzt. Es wurde spekuliert, dass die unterschiedliche Verwendung von Substraten mit der Wirtsspezifität und Virulenz von *Francisella* in Zusammenhang gebracht werden kann.

Francisella sp. Stamm W12-1067 ist ein kürzlich in Deutschland identifiziertes neues *Francisella*-Isolat, das für die typische *Francisella*-Pathogenitätsinsel FPI negativ ist. In seinem Genom ist jedoch eine alternative mutmaßliche T6SS vorhanden. Daher ist es immer noch unbekannt, ob dieser Stamm pathogen ist oder nicht. Der Metabolismus von *Francisella* ist wichtig, um in Wirtszellen replizieren zu können und ein erfolgreiches menschliches Pathogen zu sein. Daher führten wir ^{13}C-Markierungsexperimente mit *Francisella* sp. Stamm W12-1067 in komplexen Medien durch, die entweder [U-$^{13}C_6$]Glucose, [1,2-$^{13}C_2$]Glucose, [U-$^{13}C_3$]Serin oder [U-$^{13}C_3$]Glycerin enthielten. Darüber hinaus zeigte die in-silico-Genomanalyse das Vorhandensein eines mutmaßlichen Myo-Inositol-Stoffwechselweges in *Francisella* sp. Stamm W12-1067. Wir führten daher ein zusätzliches ^{2}H-Markierungsexperiment mit *Francisella* sp. Stamm W12-1067 in CDM-Medium mit myo-Inositol-C-$^{2}H_6$ durch. Durch GC-MS konnten wir zeigen, dass myo-Inositol von *Francisella* sp. Stamm W12-1067 über den myo-Inositol/Glucuronat-Stoffwechselweg und 2-Dehydro-3-desoxy-D-gluconat verwertet wird, das später in Pyruvat und Glyceraldehyd-3-phosphat für die Biosynthese von Glycogen, Protein und Fettsäuren eingebaut wird. Darüber hinaus konnten Glucose, Serin und Glycerin von *Francisella* sp. Stamm W12-1067 für die Synthese von Zucker, Protein-abgeleiteten Aminosäuren, freie Aminosäuren und freie Metaboliten verwendet werden. Wie für die anderen *Francisella*-Stämme fanden wir heraus, dass Glukose die effizienteste Vorstufe ist und dass sie über EMP, aber nicht über den ED-Weg oder PPP, in Glykogen, Aminosäuren und freie Fettsäuren, und Zellwand-Aminozucker abgebaut werden kann. Die Pyruvat-Transaminase ist auch hier nicht im Genom von *Francisella* sp. Stamm W12-1067 vorhanden. Nichtsdestotrotz konnte Ala jedoch immer noch aus Pyruvat synthetisiert werden. Serin konnte nicht für die Gluconeogenese unter den experimentellen Bedingungen benutzt werden. Dies deutet auf spezifische Substratnutzungen in einem geteilten Stoffwechselnetzwerk hin. Darüber hinaus zeigte die Myo-Inositol-Mutante von *Francisella* W12-1067 einen höheren Kohlenstofffluss zu Ala (Pyruvat) und einem niedrigeren Fluss zu Tyr. Damit scheint das Eda-Enzym in dem Wildtyp-Stamm nicht nur am Metabolismus von Myo-Inositol beteiligt zu sein. Die Abwesenheit von Eda könnte zu einer höheren Konzentration von Pyruvate und Glyceraldehyd-3-Phosphat führen, da in der Mutante kein Kohlenstofffluss zu 2-Dehydro-3-desoxy-D-gluconat vorhanden ist.

Bakterielle Biofilme sind durch die Bildung einer extrazellulären Matrix gekennzeichnet, die aus

extrazellulären Polysacchariden, Proteinen und manchmal auch DNA besteht. Diese Matrix kapselt die Zellen in dreidimensionalen Architekturen ein. Insbesondere trägt diese schützende Umgebung auch zu verringerten Aktivitäten von antibakteriellen Arzneimitteln und somit zu mehr Resistenzen bei der Chemotherapie bakterieller Infektionen bei. Als am besten untersuchtes Gram-positives Bakterium dient *Bacillus subtilis* als ausgezeichnetes Modell zur Untersuchung der metabolischen Basis der Biofilmbildung. SinR ist der Hauptregulator von Biofilm-Genen. Er wirkt durch Bindung an die Promotorregionen der *tapA-sipW-tasA* und der *epsA-O* Operons, die das extrazelluläre Amyloid bzw. die Enzyme für die extrazelluläre Polysaccharidsynthese kodieren. Es wurde auch gezeigt, dass die Phosphodiesterase YmdB für die Expression von Biofilm-Genen benötigt wird. In dieser Arbeit wurden nun Markierungsexperimente von drei *B. subtilis*-Mutanten durchgeführt, die unter planktonischen Bedingungen oder auf Agarplatten wuchsen, wobei [U-$^{13}C_6$]Glucose oder [U-$^{13}C_5$]Glutamat als Vorstufe verwendet wurden. Insbesondere wurden der undomestizierte Stamm NCIB 3610, die Δ*ymdB*-Mutante des NCIB3610-Stammes (GP921), der hinsichtlich der Biofilmbildung defekt ist, und die Δ*sinR*-Mutante des NCIB3610-Stammes (GP1562) untersucht. Für die planktonischen Experimente konnte belegt werden, dass ΔSinR und ΔYmdB keine signifikante Rolle spielten. Auf der Agarplatte wurden jedoch signifikante metabolische Unterschiede zwischen den drei Mutanten gefunden, die sich auf die Bildung des Biofilms (extrazelluläres Polysaccharid mit Aminosäureketten) beziehen könnten.

Braunes Fettgewebe (BAT) findet sich hauptsächlich im Hals und Thorax. Dessen Menge hängt von verschiedenen Faktoren ab, wie Alter, Geschlecht, Umgebungstemperatur etc. Das Entkoppelnde Protein 1 (UCP1), das in der inneren Membran der Mitochondrien gefunden wird, spielt eine zentrale Rolle im Thermogenese-Prozess von BAT. UCP1 kann durch freie Fettsäuren aktiviert werden, die von Triacylglycerolen durch eine Lipase abgeleitet sind und durch Purinnukleotide inhibiert werden können. Bis jetzt ist die Funktion von UCP1 im Zusammenhang mit dem Stoffwechsel noch unbekannt. Isoproterenol könnte in BAT Fettsäuren freisetzen, die das UCP1 aktivieren. Wir führten Markierungsexperimente mit „immortal“ BAT-Zellen durch, die in DMEM-Medium wuchsen, das [U-$^{13}C_6$]Glucose enthielt. Während der Kultivierung wurde Isoproterenol hinzugefügt, um das UCP1 zu aktivieren. Die Markierungsdaten belegten, dass nach der Aktivierung (i) der Glucosefluss in die Glykogenspeicherung erhöht ist, (ii) mehr Lactat aus Glucose erzeugt wird, und (iii) der Kohlenstoffffluss in den oxidativen TCA-Zyklus geleitet wird, wahrscheinlich um mehr Energie zu erzeugen.

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1. Introduction

1.1 Metabolism and its analysis methods

Metabolism is the assembly of chemical transformations in cells or organism which maintain the life continuing. The metabolism process can be divided into 2 classes: catabolism and anabolism, which represent the breaking down and the building of components in the cells or organisms. Generally, catabolism process releases energy and, in opposite, the anabolism process consumes energy. Here, enzymes play significant roles in the metabolism process, which accelerate the chemical reactions. As regulators of metabolic pathways, they can respond to changes of environment in cells or to signals from other cells.

The different metabolic pathways include catabolic and anabolic reactions. Under catabolic reactions, the carbohydrate or fats can be converted into energy, which can be used in the anabolic reactions for the synthesis of macromolecules and the other necessary compounds. The energy supplied from food or light can be stored into adenosine triphosphate (ATP). Afterwards, during ATP hydrolysis, the necessary energy can be released, to drive energetically unfavourable reactions. The catabolic energy conversion can be divided into three stages, at the first stage, the macromolecules from the ingested food are broken down into smaller units and no appreciable energy is produced, for example, the degradation of fat into fatty acids and glycerol; the degradation of polysaccharide into glucose and the degradation of protein into amino acids. At the second stage, the small units are further degraded into key metabolites such as acetyl-CoA. During this stage, small amounts of ATP are produced. At the third stage, the tricarboxylic acid (TCA) cycle is active and abundant ATP is generated by the complete oxidation of acetyl-CoA. For instance, when glucose is degraded into water and CO_2, 30 ATP are generated (**Figure 1**, Berg et al., 2010). Intermediates from the second catabolism stage (e.g. oxaloacetate (OAA), α-ketoglutarate) play a central role in the metabolism of all organisms. For example, from OAA, Asp, Asn, Thr, Lys, Met, pyrimidine and NADH can be formed; from α-ketoglutarate, Glu, Gln, Arg and Pro can be formed.

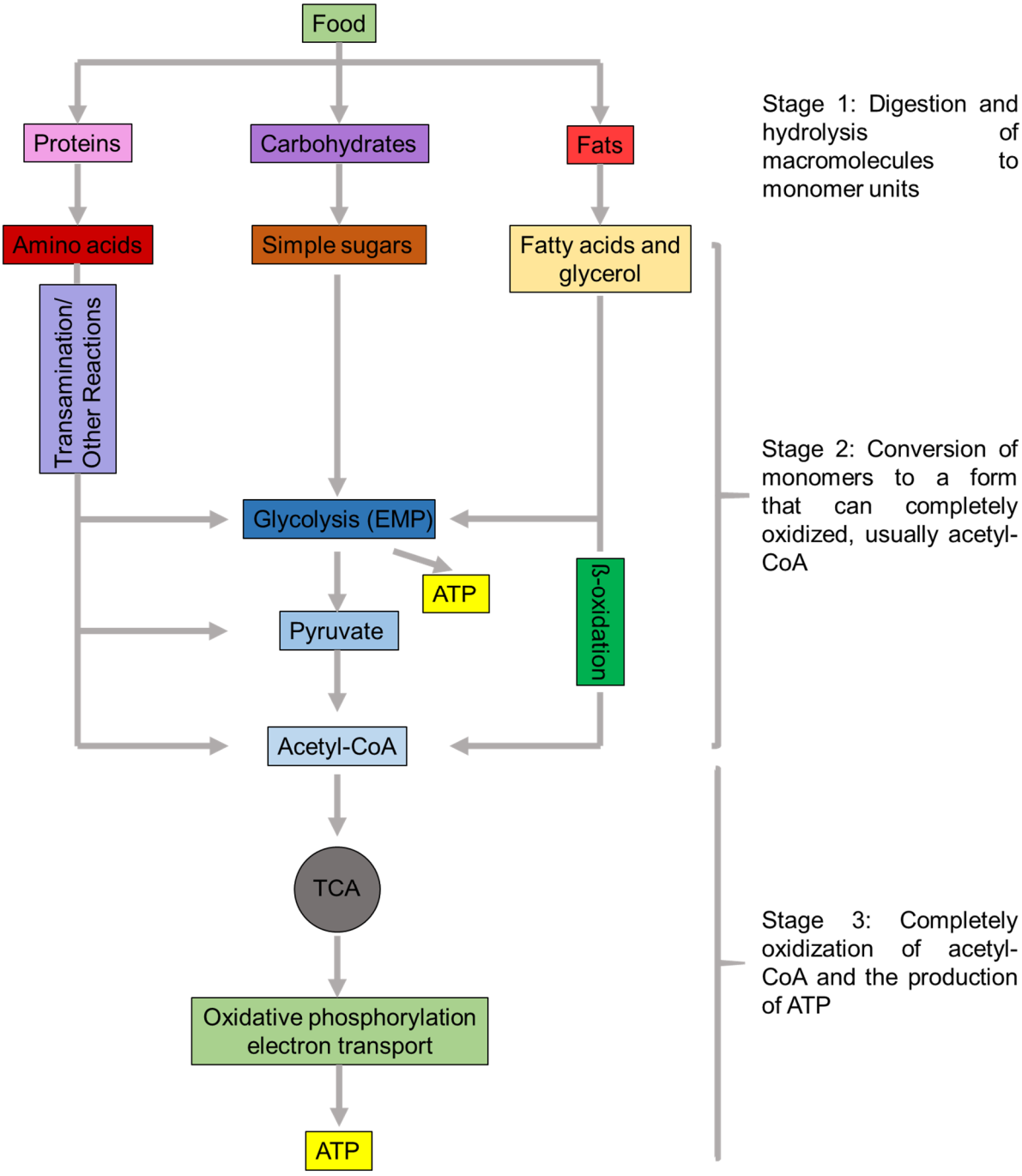


Figure 1. The catabolic energy conversion stages (modified from Bruice, 2016)

1.1.1 Isotopologue profiling

The isotopologue profiling analysis enables analysing the metabolic flux and biosynthesis pathways of metabolic intermediates or final products (Eylert et al., 2010; Eisenreich et al., 2013). With the help of this technique, problems like the degradation pathway of substrate, the biosynthesis of different metabolites and the relation between the metabolism and pathogeny can be solved. Briefly, this method is using the isotope tracer as a substrate to cultivate different organisms. Subsequently, different metabolites are isolated from the organisms and analysed. For example, the percentage of

stable carbon isotope ^{13}C is only 1.1 % in nature, after the labelling experiment using a ^{13}C-enriched precursor as a substrate, these abundances are modulated and the ^{13}C-enrichment and the isotopologue distribution of multiple metabolites (e.g. amino acids, polar metabolites, sugars etc.) can be analysed by using MS or NMR (Eylert et al., 2009; Gillmaier et al., 2012).

1.1.2 Metabolomics analysis

Metabolomics is the quantitative and qualitative analyse of all metabolites in a biological system, which represent the total biosynthesis and the global dynamic response of a cell/organism to its stimuli. With the help of metabolomics, the interaction between environment and genes in an organism can be analysed. Moreover, metabolic phenotypes can also be characterized (Fiehn 2001; Ryan and Robards 2006; Oliver et al. 1998).

The metabolisms of different organisms are very complex considering that there are about 3,000 (in humans) to over 200,000 (in plants) metabolites ("metabolomes"). In order to determine the metabolomes of different organisms, various methods have been developed like metabolites profiling, target analysis, metabolites fingerprinting and metabolite footprinting (see also **Table 1**, Blow 2008; Hartmann et al. 2005; Ryan und Robards 2006; Allen et al. 2003; Madsen et al. 2010).

Table 1. Different analysis in metabolomics

Metabolomics analysis	Identification and quantification of the total metabolome in a biological system
Metabolite profiling	Identification and quantification of a defined set of metabolites belonging to a particular metabolic pathway or to a specific class of substrates
Targeted analysis	Qualitative and quantitative analysis of a specific metabolites or a very limited number of metabolites
Metabolite fingerprinting	Quick classification of many samples without identification and quantification of certain metabolites
Metabolite footprinting	Similar as fingerprinting analysis, however, only extracellular metabolites are analysed

1.2 *Francisella* strains

1.2.1 *Francisella* strains and tularmia disease

Francisella tularensis (*Ft*) is an intracellular Gram-negative pathogenic bacterium which can cause infective *tularemia* disease in many animals including humans (Ellis et al., 2002; Sjostedt, 2011). *Ft* can infect approximately 250 wildlife species (including mammals, rodents, ticks and other anthropods) (Foley and Nieto, 2010; Santic et al., 2010). *Tularemia* infects mostly via aerosol ingestion or skin inoculation. Because of the high pathogenicity of *Ft*, it is registered as a biological weapon (Dennis et al., 2001). About 10-20 *Francisella* subsp. *tularensis* (*Ftt*, *Ft*-type A, mainly found in North America) are infective and can cause lethal *tularemia* (Ellis et al., 2002). Another *Ft*-type, *Ft holarctica* (*Ft*-type B) is also highly pathogenic and infectious. It can be found throughout the Northern hemisphere. This strain can also be found in Europe in infected animals and humans. Another mouse-pathogenic *Francisella* strain *F. novicida* (*Fno*) can be classified as *F. tularensis* subspecies *novicida* or as a separate species (Busse et al., 2010; Johansson et al., 2010; Kingry and Petersen, 2014). In this work, it is designated as *Fno*.

Ft-type A and *Ft*-type B (*Ftt* and *Fth*) replicate in the host cells' cytosol, especially in the cytosol of macrophages (Santic et al., 2006; Sjostedt, 2006; Keim et al., 2007; Jones et al., 2012). After the phagocytosis of macrophages, the pathogens enter into the macrophages and form the *Francisella*-containing phagosomes (FCPs). The FCPs then activate the *Francisella* pathogenicity island (FPI). FPI encodes a type 6 secretion system (T6SS), which enables the entering of *Ft* into the cytosol of the host cell (Chong et al., 2008; Clemens et al., 2015; Rigard et al., 2016). To replicate successfully in this niche, the efficient utilization of nutrients from this environment is necessary (e.g., for energy generation and biosynthesis purposes). However, the host cell's cytosol does not provide a rich enviorment for nutrients for mutiplication as show by injection experiments with *Salmonella typhimurium* (Goetz et al., 2001). Therefore, some pathogenic bacteria including *Francisella*, *Listeria monocytogenes*, *Shigella*, and *Rickettsia* spp. have to adapt their metabolism for multiple substrate usage (Santic and Abu Kwaik, 2013; Eisenreich et al., 2017). More specifically, *L. monocytogenes* utilizes multiple substrates (e.g., glucose phosphate, glycerol and amino acids) which are shuffled into a bipartite metabolic network optimized to serve for specific metabolic traits (Grubmüller et al., 2014). With the help of multiple substrates, intercellular bacteria can also divide in phagosomal compartments (Abu Kwaik and Bumann, 2013, 2015; Schunder et al., 2014; Eisenreich and Heuner, 2016; Gillmaier et al., 2016; Häuslein et al., 2016).

However, in *Francisella* strains the understanding of the relationship between nutrient usage and life cycle is still unclear (Checroun et al., 2006; Meibom and Charbit, 2010; Santic and Abu Kwaik, 2013;

Barel et al., 2015). Currently, it was demonstrated that *Francisella* strains use multiple substrates including amino acids (e.g., serine, glycine, cysteine, glutamate, glutamine, asparagine), small peptides and other gluconeogenetic substrates like glycerol or glycerol phosphate in some accordance to *Listeria* (**Figure 2**) (Alkhuder et al., 2009; Raghunathan et al., 2010; Brown et al., 2013; Gesbert et al., 2013, 2015; Barel et al., 2015; Brissac et al., 2015; Ramond et al., 2015). Several major facilitator superfamily (MFS)-type and amino acid-polyamine-organo cation (APC)-type uptake systems for amino acids were found in the genomes of *Ft* strains also confirming the potential usage of amino acids (Alkhuder et al., 2009; Meibom and Charbit, 2010). Recently, Gesbert and Ramond characterized some of these transporters in detail (Gesbert et al., 2014, 2015; Ramond et al., 2014, 2015). Earlier work showed that serine was a main carbon substrate for intracellularly multiplying *Francisella* strains (Meibom and Charbit, 2010; Raghunathan et al., 2010; Barel et al., 2012; Gesbert et al., 2013, 2015; Brown et al., 2014), Barel and his coworkers confirmed this that the SLC family of host amino acid transporters is important for intracellular replication of Fth strain LVS (Barel et al., 2012). Furthermore, Brown and his co-workers mentioned that the glycine cleavage system and the glycine dehydrogenase are also important for replication in serine limiting environments (Brown et al., 2014). In addition, serine is important for *Fth* growth under in vitro conditions, although *Ft* is not auxotroph for Ser (Meibom and Charbit, 2010; Brown et al., 2014).

Similar as *L. monocytogenes*, in some *Francisella*'s sequenced genomes glycerol-3-phosphate dehydrogenase and transferase were found. On this basis, glycerol (and its phosphate) could serve a potential nutrient source at least for some *Francisella* species and *Ft* strains. Huber and Brissac found that *Ftt*, *F. hispaniensis*, F. *philomiragia*, and *Ft* subsp. *mediasiatica* could indeed metabolize glycerol (Huber et al., 2010; Brissac et al., 2015). However, *Fth* strains could metabolize glycerol phosphate, but not glycerol (Gyuranecz et al., 2010).

Comparing to *L. monocytogenes*, *Ft* has neither glucose phosphate uptake system (such as UhpT, nor a putative glucose transporter system (PTS-type and non-PTS-type). Nevertheless, some *Francisella* strains could still metabolize glucose. Gyuranecz and Gesbert found that *Fth* isolates utilized glucose but not glucose 6-phosphate (Gyuranecz et al., 2010; Gesbert et al., 2014). *Fno* could metabolize cellobiose, galactose, and sucrose (Huber et al., 2010). From the genome data, all of these sugars seem to be metabolised by the glycolytic pathway (EMP pathway) and/or the non-oxidative pentose phosphate pathway (PPP), but not via the Entner-Doudoroff pathway (ED) and the oxidative branch of the PPP. To produce energy, NADH production via a complete citrate cycle (TCA) appears to be important. Anaplerotic reactions could then interconnect the EMP pathway with the TCA. A schematic overview of the key metabolic pathways is given in **Figure 2**.

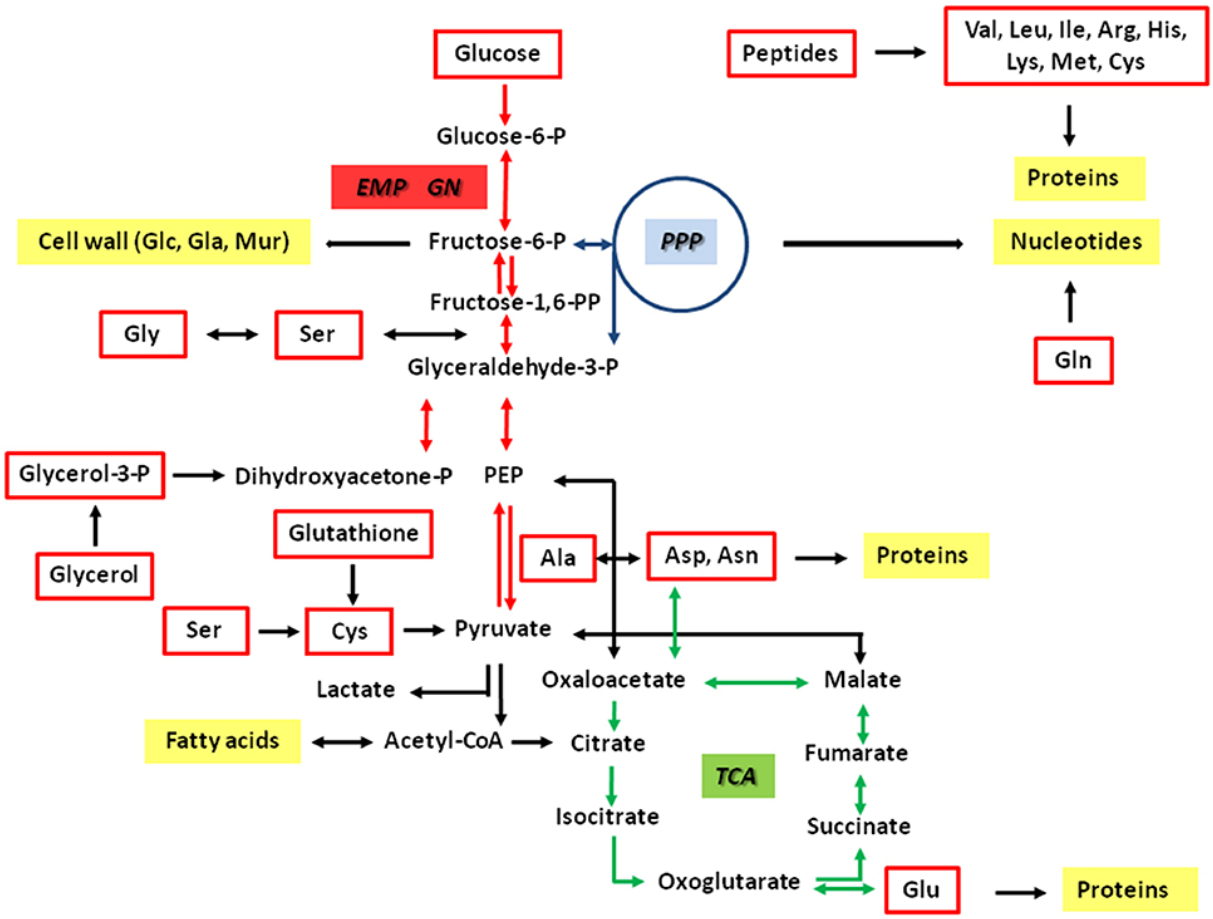


Figure 2. Hypothetical metabolic network of *Francisella tularensis*. The pathways are predicted on the basis of the genome sequences and earlier biochemical studies (Gyuranecz et al., 2010; Meibom and Charbit, 2010; Raghunathan et al., 2010; Barel et al., 2015). Potential carbon substrates are indicated by red boxes. Major metabolic products are indicated by yellow boxes. Reactions of the glycolytic pathway (EMP) and gluconeogensis (GN) are indicated by red arrows. Reactions of the pentose phosphate pathway (PPP) and the citrate cycle (TCA) are indicated by blue and green arrows, respectively. The interconnection between Gly and Ser includes the glycine cleavage system.

Therefore, *Ft* comes as a bacterium to be able to metabolize different substrates. However, from the unusually high amount of disrupted biosynthetic pathways in *Ftt* Schu S4, it was suggested that *Ftt* is an obligate host-dependent bacterium in its natural life cycle (Larsson et al., 2005; Meibom and Charbit, 2010). Take together, the details of metabolite usages and fluxes in *Ft* and the relationship between the virulence of different *Francisella* species and the usage of substrates are still obscure.

1.2.2 *Francisella* W12-1067 and the myo-inositol metabolism

Recently, a new *Francisella* species (*Francisella* sp. strain W12-1067) was identified in Germany (Rydzewski et al., 2014). The genome DNA sequence of this strain is 89% identical to the respective nucleotide sequence of the recently published strain *F. guangzhouensis* (Gu et al., 2016). However, whereas *Francisella* strain W12-1067 is not identical to *F. guangzhouensis*, it was also isolated from a

water reservoir of a cooling tower (Rydzewski et al., 2014). Notably, strain *F.* W12-1067 is also able to persist in a human derived macrophage-like cell line, but its virulence for humans or mice is not known yet. The draft genome was annotated and in silico analysis identified the presence of various virulence genes common to the genus *Francisella*, but the *Francisella* pathogenicity island (FPI) is missing. However, another putative alternative type-VI secretion system (T6SS) is present within the genome of strain W12-1067 (Rydzewski 2014).

Myo-inositol (MI) is abundant in soil and common in plants and animals (Turner et al., 2002). Various different bacteria are able to grow on MI as the sole carbon source (Yoshida et al., 2007; Lim et al., 2007). The phosphorylated form (inositol hexakisphosphate) is also called phytate. Phytate is an important carbon and phosphorus storage compound in plants and seeds and can be degraded by the enzymatic activity of phytases to myo-inositol and free phosphate (Lim et al., 2007; Rao et al., 2009). Some bacteria use a myo-inositol oxygenase (glucuronic-utilizing pathway) to convert it to glucuronic acid and, in further steps, to glyceraldehyde 3-phosphate and pyruvate (**Figure 3**, Kilgore and Starr 1959; Ashwell et al., 1960; Peekhaus and Conway, 1998).

In *F*. W12-1067, there seems to be the potential to degrade MI (on the basis of genome sequence data), but experimental evidence for the functionality of this pathway is missing.

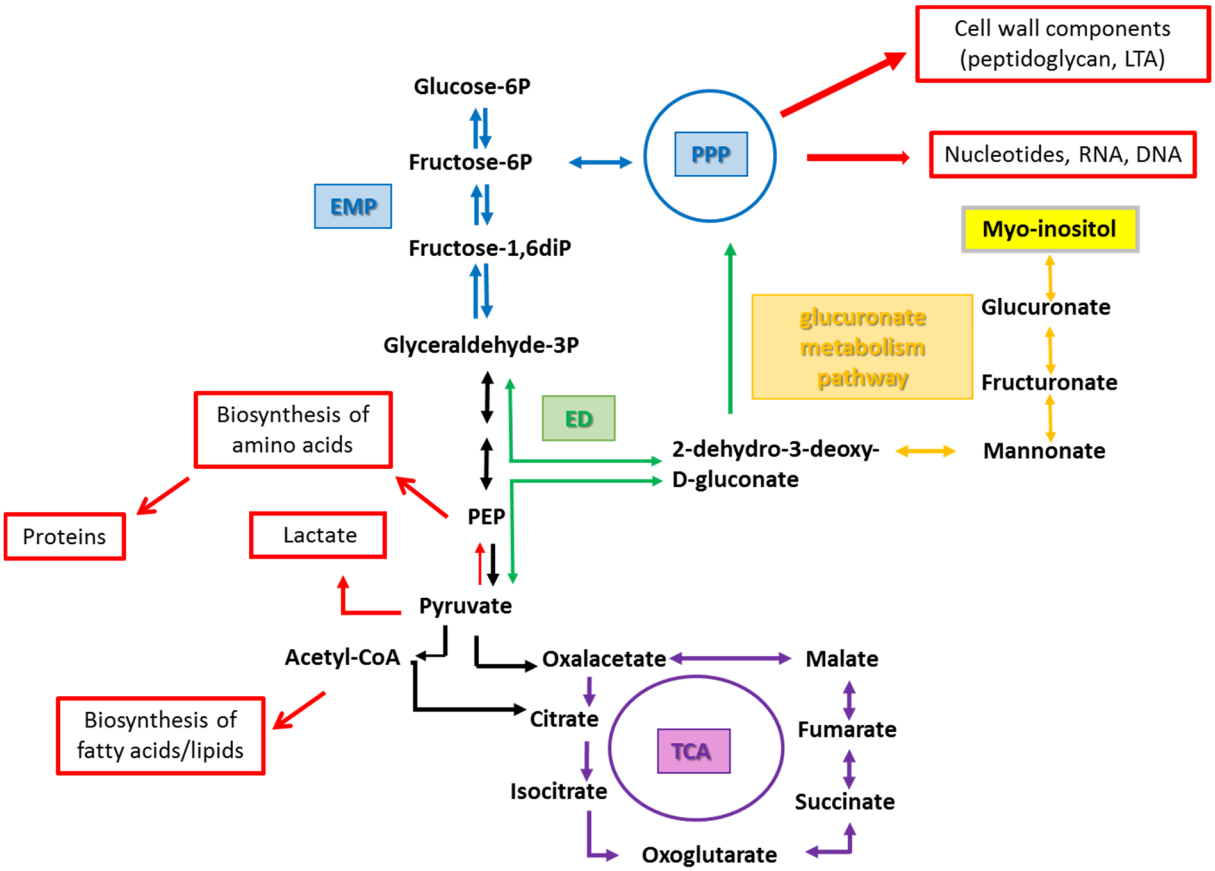


Figure 3. Hypothetical myo-inositol degradation pathway of *Francisella* W12-1067. The pathways are predicted on the basis of the genome sequences and earlier biochemical studies (Rydzewski et al., 2014).

1.3 Biofilm formation in *Bacillus subtilis*

1.3.1 General aspects and its formation in *Bacillus subtilis*

Biofilm is defined as a bacterial cell community which is surrounded by a self-produced polymer matrix. This matrix stands mostly on the bacterial surface, which contains polysaccharides, protein, lipids and extracellular DNA (Hall-Stoodley, 2012). Anthony van Leeuwenhoek (1632-1723) found aggregated microbes in his mouth using a primitive microscope (Dobell et al., 1960). About 100 years later, Louis Pasteur (1822-1895) thought that aggregates of bacteria as the cause of wine and drew it (Pasteur, 1864; Hoiby, 2014). In 1922, EC. Angst found that the slime on the ships was caused by bacteria. ZoBell then studied the adherence and growth of bacteria on submerged glass slide in seawater, and found that the biofilm consist of bacteria (ZoBell et al., 1935). One of the first statements about biofilm in medicine was published in 1977, showing an image of of aggregated bacterial cell surrounded by slime from the lungs of a cystic fibrosis patient (Hoiby, 1974). In 1981, J.W. Costerton and his coworker published electron microscopy of *P. aeruginosa* microcolonies, in which he described the bacterial “glycolyx” (Costerton et al., 1981). Six years later, he published

another work, in which he replaced the “glycolyx” with “biofilm” (Costerton et al., 1987). The first two medical reports using the word “biofilm” were published in 1981 by Jendresen and his coworkers (Jendresen et al., 1981; Jendresen et al., 1981). After these reports, the term “biofilm” was broadly accepted and used to describe the phenotype of sessile bacteria. In 1996 J.W. Costerton hosted the first ASM biofilm conference in Snowbird, Utah, USA, establishing a new research field (Hoiby, 2014).

As a model Gram-positive bacterium *Bacillus subtilis* has been studied in different research areas. The first report of biofilm formation in *B. subtilis* was published in 2001 by Branda and his coworkers (Branda et al., 2001). In this work, a picture of floating biofilms on the top of MSgg medium surface was shown (**Figure 4**). The bacterial cells were embedded in a self-produced extracellular matrix, which contained mainly polysaccharides, protein, nucleic acids and lipids (Marvasi et al., 2010; Cairns et al., 2014). The 15 genes epsA-O (eps) control the biosynthesis and export of exopolysaccharides. A mutant lacking these genes, can’t form colony and pellicle (López et al., 2010). However, the single gene deletion has not been studied till now (Blair et al., 2008; Guttenplan et al., 2010). The tapA-sipW-tasA (tapA) operon encodes the proteins in this extracellular matrix. The deletion of these genes decreased the colony structure and pellicles formation (Branda et al., 2006).

Figure 4. Pellicle formation of the undomesticated *Bacillus subtilis* wild type NCIB3610 on the top of MSgg medium surface (Branda et al., 2001).

1.3.2 The *SinR* protein and the *YmdB* protein

The *SinR* protein can regulate the biofilm gene expression and the switch between a sessile life style and motility. In the beginning, it was studied because of the flagella-less and non-motile phenotype (Fein, 1979; Pooley & Karamata, 1984; Sekiguchi et al., 1990). Later, because of its regulation for biofilm formation, the *SinR* protein was more and more studied (Kearns et al., 2005). Kearns also demonstrated that the eps operon, which controls the exopolysaccharide formation, is under the control of *SinR* protein. Moreover, it was shown that mutations within the gene encoding the *SinR*

protein can restore wild type like biofilm formation (**Figure 5**).

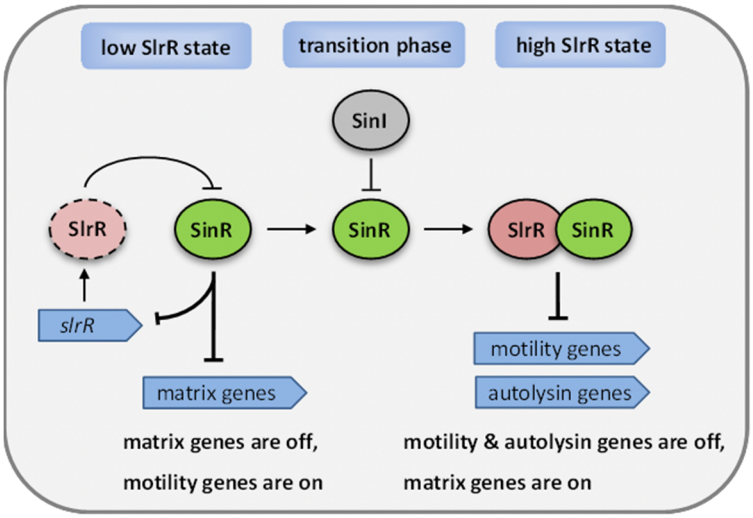


Figure5. A bistable switch of the *SinR* and its antagonists controls cell differentiation (modified from Vlamakis et al., 2013).

The *ymdB* deletion mutant of *B. subtilis* showed a strong overexpression of a certain protein in an SDS-PAGE analysis of cell extracts. This overexpressed protein could be identified as Hag, the flagellin protein (Diethmaier et al., 2011). In addition, the *ymdB* gene deletion mutant showed strong defects in biofilm formation. Colonies formed on biofilm-inducing MSgg agar plates appeared smoothly and shinyly compared to the rough colonies of the wild type strain (**Figure 6**).

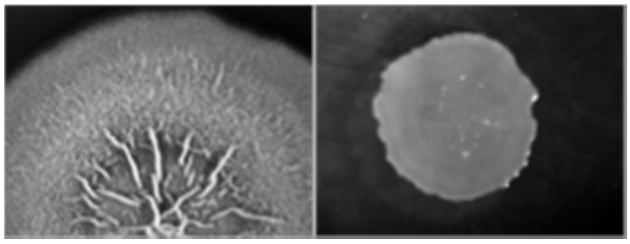


Figure 6. Phenotype of WT (left) and the Δ*ymdB* mutant strain (right). Loss of the *ymdB* phosphodiesterase leads to a drastically reduce colony structure on biofilm inducing MSgg agar plates (Diethmaier et al., 2011).

1.4 Immortal brown adipose tissue and the isoproterenol activation

1.4.1 Brown adipose tissue and uncoupling protein 1

Usually, the adipose tissue in mammals is divided into two different groups: white adipose tissue (WAT) and brown adipose tissue (BAT). White adipose tissue stores energy while brown adipose tissue catabolizes lipids mostly to produce heat (Gesta et al., 2007). The large number of innervated

and vascularized brown adipose tissues in mitochondria shows a reddish brown colour. Thus, it is so called brown adipose tissue. BAT locates mainly in neck and thorax, whose amount depends on various factors, such as age, gender, environmental temperature, etc. (Cypess et al 2009). Uncoupling protein 1 (UCP1), which was found in the inner membrane of mitochondria, plays a central role in the thermogenesis process of BAT (Cannon and Nedergaard, 2004). To decrease the proton gradient, UCP1 increases the permeability of the inner mitochondrial membrane and allows protons, which have been pumped into the intermembrane space, return to the mitochondrial matrix without driving the ATP synthase. The uncoupling of the respiratory chain after the activation of UCP1 allows a fast oxidative metabolism with slow ATP synthesis (Cousin et al., 1992; Guerra et al., 1998; Himms-Hagen et al., 2000; Xue et al., 2007; Young et al., 1984). UCP1 can be activated by free fatty acids which derived from triacylglycerols by a lipase and can be inhibited by purine nucleotides. Fatty acids cause the release of norepinephrine from sympathetic nervous system to activate the adenylyl cyclase when it combines with the beta-3 adrenergic receptor on the plasma membrane. That procedure indicates cyclic AMP (cAMP) formation from ATP resulting in the activation of protein kinase A. Later, the phosphorylated triacylglycerol lipase is active by the activated protein kinase A. UCP1 will be inactivated because of the heat generated from the thermogenesis leading to the cell back to the normal state (see **Figure 7**).

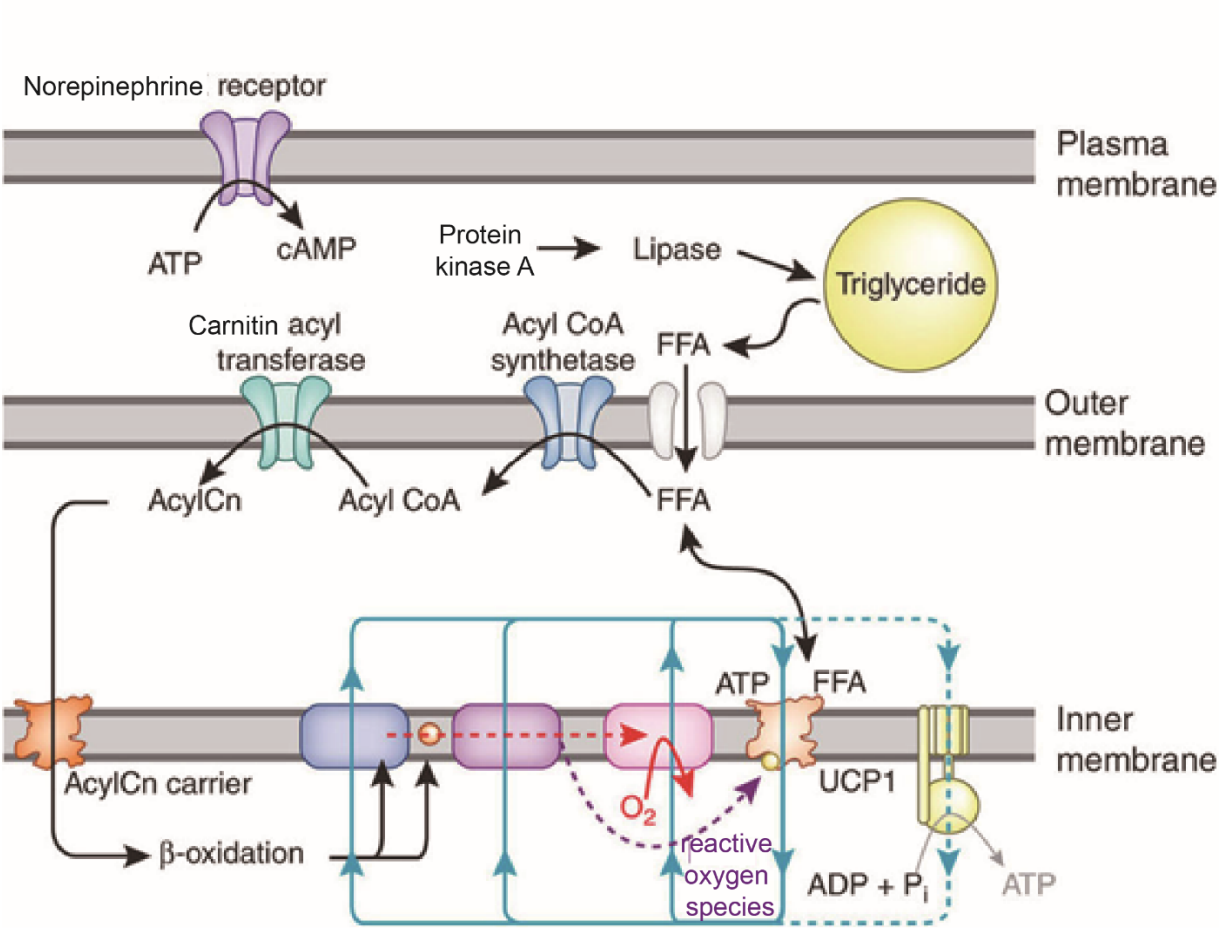


Figure 7. The canonical mechanism for the activation of UCP1 and thermogenesis by free fatty acids (FFA) in brown adipose tissue (modified from Nicholls and Rial, 2016).

1.4.2 Isoproterenol and its function in the BAT cells

Isoproterenol or isoprenalin (**Figure 8**) is a structural isomer of orciprenaline, a synthetic racemic norepinephrine derivative used as a sympathomimetic. This drug was patented in 1943 by Boehringer Ingelheim. Isoproterenol is an adrenaline-like substance which activates β-1 and β-2 adrenergic receptors, however, has no activity against the α-adrenergic receptor. Its N-isopropyl substituent is responsible for this selectivity (Mozayani et al., 2003). It can cause the relaxation of the bronchial and vascular musculature, and the increase of the contraction force, heart rate and blood pressure. This effect on heart occurs through the stimulation of the β-1 adrenoceptors. Via the receptors, the enzyme adenylyl cyclase is activated, which catalysts the synthesis of cyclic adenosine monophosphate (cAMP). Due to the increased cAMP concentration, the enzyme protein kinase A is activated which leads to the phosphorylation of the voltage-dependent calcium ion channels. That is conductive to the increase of Ca^{2+} concentration during the cell depolarisation, which leads to an increased heart rate. In addition, isoproterenol can slow down the antigen-induced release of the messenger histamine, which leads to the slowing down of the anaphylaxis mediation and the increase of the lactate production (Larsen 1999). In brown adipose tissues isoproterenol can upregulate UCP1 and thermogenesis since it is a non-specific AR agonist. This property has been shown in vivo and in vitro with primary BAT cells (Li et al., 2014; Beattie et al., 2000).

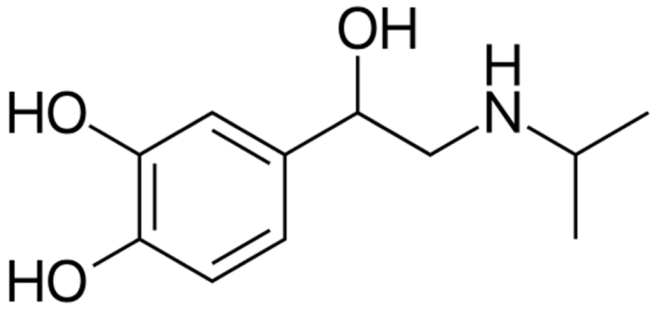


Figure 8. Structure of isoproterenol

1.5 Aims of the thesis

The aim of this PhD thesis was to study metabolic pathways and fluxes in different system (pathogenic bacteria, bacteria producing biofilm and BAT) using ^{13}C-labeling experiments and isotopologue profiling. In all of these biological systems, the rates and the pathways of glucose usage was unclear, although glucose was considered as a main carbon substrate in all of these systems. Moreover, the

glucose pathway seemed to contribute to carbon allocation and downstream signalling forming phenotypes that are specific for these cells and organisms (i.e. biofilms in the *B. subtilis* project, pathogenicity in the *Francisella* project, and heat formation in the brown fat cells). It appeared therefore in order to comprehensively study these systems in this thesis. Briefly, ^{13}C-labeled substrates such as glucose, glutamate, glycerol and serine were supplied in the liquid medium or on agar plates. Due to the fluxes in the core metabolic networks, specific ^{13}C-enrichments and isotopologue compositions were generated in downstream products (e.g. amino acids, fatty acids and sugars) which could be determined at high sensitivity by gas chromatography/mass spectrometry (GC/MS). By comparison of these patterns, the pathways involved in the metabolism of the supplemented tracers including glucose could be reconstructed for the various bacteria and eukaryotic cells in considerable details.

In the first part (Result 3.1), we studied the differential substrate usages and metabolic fluxes in *Francisella tularensis* subspecies *holarctica* (WT isolated from beaver and LVS) and *Francisella novicida*. To investigate the different usages, we added [U-$^{13}C_6$]glucose, [1,2-$^{13}C_2$]glucose, [U-$^{13}C_3$]serine, or [U-$^{13}C_3$]glycerol as isotope tracers to the complex medium T. After the labelling experiments, protein derived amino acids, polar metabolites and sugars were isolated from the dried bacterial cell pellets and analysed using GC/MS. Our finding confirmed multiple substrate usage of *Francisella* strains with glucose as a main carbon substrate. The fluxes, however, differed for the strains under study indicating that the metabolic differences of *Fth* strains and *Fno* could be related to their host-specific virulence.

In the second part (Result 3.2), we investigated the differential substrate usage and metabolic fluxes in *Francisella* sp. strain W12-1067 (new environmental isolated), providing similar fluxes as in Result 3.1. However, we also analysed a Δmyo-inositol mutant of this strain using [U-$^{13}C_6$]glucose and [U-$^{13}C_3$]glycerol as isotope tracers. Owing to the knockout of the myo-inositol pathway, higher fluxes from glucose via the EMP pathway could be determined. Using ^{2}H-labelled inositol, we could then show that exogenous inositol was indeed incorporated and used as a general substrate to feed the PPP and the downstream pathways leading to glucogen and protein biosynthesis.

In the third part (Result 3.3), the metabolism of *Bacillus subtilis* growing under planktonic condition or on agar plate, was studied for different mutants using [U-$^{13}C_6$]glucose or [U-$^{13}C_5$]glutamate. More specifically, the undomesticated strain NCIB 3610, the Δ*ymdB* mutant of NCIB3610 strain (GP921),

which is defective in biofilm formation, and the Δ*SinR* mutant of NCIB3610 strain (GP1562) were investigated. Δ*SinR* controls the biofilm formation. For the planktonic experiments, without a surface for the biofilm formation, it was shown that Δ*SinR* and Δ*ymdB* didn't play a significant role in directing the metabolic fluxes. However, on the agar plate, distinct metabolic differences between the three strains were discovered, which could relate to the biofilm (extracellular polysaccharides with amino acid chains) formation.

In the last part (Result 3.4), the metabolism during isoproterenol activation in immortal brown adipose tissue (IBAT) cells was analysed. We performed labelling experiments with IBAT cells growing in DMEM medium in the presence of [U-$^{13}C_6$]glucose with or without isoproterenol. The results presented that during the isoproterenol treatment, glucose was directly stored in the glycogen and the energy was generated via pyruvate to acetyl-CoA by losing CO_2.

2. Materials and method

2.1 Materials

2.1.1 Laboratory Equipment

Table 1

Item	Description	Manufacturer
Benchtop centrifuge	A-14	Jouan GmbH (Unterhaching, Germany)
Centrifuge	Biofuge Primo R	Heraeus (Traunstein, Germany)
Drying oven	E28	Binder GmbH (Tuttlingen, Germany)
Freeze-dryer	Alpha 2-4 LD plus Vacuum pump: RC 5	Christ (Osterrode, Germany) Vacuurbrand GmbH & Co. KG (Staufen, Germany)
GC/MS	Gas Chromatograph GC-2010 Mass Spectrometer QP-2010 Auto Injector AOC-20i Auto Sampler AOC-20s GC Column: Equity™-5, FUSED SILICA Capillary Column, 30 m x 0.25 mm x 0.25 µm film thickness	Shimadzu (Neufahrn, Germany) Shimadzu (Neufahrn, Germany) Shimadzu (Neufahrn, Germany) Shimadzu (Neufahrn, Germany) SUPELCO (Bellefonte, USA)
Glass beads	0.25 – 0.55 mm	Roth (Karlsruhe, Germany)
Heating block	Techne DRI-Block® DB 2A	Thermo-DUX GmbH (Wertheim, Germany)
Magnetic stirrer	MR Hei-Standard	Heidolph (Schwabach, Germany)

Micro scales	VWR-503B, (0.001 g – 500 g)	VWR (Radnor, USA)
Ribolyser		Hybaid (Kalletal, Germany)
Rotary evaporator	Rotavapor-R Diaphragm vacuum pump Water bath	Büchli (Flawil, Switzerland) Vacuubrand GmbH & Co. KG (Wertheim, Germany) Heidolph (Schwabach, Germany)
Thermostat	IKATRON® ETS-D4 fuzzy	IKA-Werke GmbH & Co. KG (Staufen, Germany)
Ultrasonic bath	USC 300T	VWR (Radnor, USA)
Vortex mixer	Reax 2000	Heidolph Elektro GmbH & Co. KG (Kelheim, Germany)

2.1.2 Software

Table 2

Software		**Manufacturer**
Adobe Illustrator	Adobe Illustrator CS4	Adobe Systems GmbH (Munich, Germany)
ChemOffice 2015	ChemDraw Professional 15.0 ChemFinder 15.0	CambridgeSoft (Massachusetts; USA)
GCMS Solution	GCMS Analysis Editor GCMS Postrun Analysis GCMS Real Time Analysis	Shimadzu Corporation (Kyoto, Japan)
Microsoft Office 2013	Excel 2013 Word 2013 PowerPoint 2013	Microsoft (Redmond, USA)

OriginPro 2017	OriginPro 2017(64-bit)	OriginLab Corporation (Northampton, USA)

2.1.3 Chemicals

Labelled precursors ([U-$^{13}C_6$]glucose, [U-$^{13}C_3$]glycerol, [U-$^{13}C_3$]serine, [1,2 -$^{13}C_2$]glucose and [U-$^{13}C_5$]glutamate) were received from Isotec/Sigma-Aldrich (St. Louis, USA) or Cambridge Isotope Laboratories (Tewksbury, Massachusetts, USA).

Further chemicals used in this work were received from AppliChem GmbH (Darmstadt, Germany), BD Biosciences (Franklin Lakes), Bio-Rad (Munich, Germany), Eppendorf (Hamburg, Germany), Merck (Darmstadt, Germany), Carl Roth GmbH & Co. KG (Karlsruhe, Deutschland), Sigma-Aldrich (St. Louis, USA), Thermo Fisher Scientific (Waltham, USA) and VWR (Radnor, USA).

All the solvents used were at least of HPLC grade.

2.2 Methods

2.2.1 *Francisella* samples

2.2.1.1 Cultivation of *Fransicella* strains

These cultivation experiments were done in cooperation with Kerstin Rydzewski and Kristin Köppen at the Robert Koch Institute (Berlin) under the supervision of PD Dr. Klaus Heuner.

2.2.1.1.1 Strains, Growth Conditions, Media and Buffers

Fno strain U112 (ATCC 15482; Larson et al., 1955), *Fth* strain LVS (ATCC 29684), *Fth* wild type strain (“Beaver” isolate; Ft-type B) (Schulze et al., 2016), *Francisella* W12-1067 WT and its Δmyo-inositol mutant (Rydzewski et al., 2014) were used. *Francisella* strains were cultivated in medium T (Pavlovich and Mishan'kin, 1987; Becker et al., 2016) containing 1% brain heart infusion broth (Difco Laboratories, Inc., Sparks, MD, USA), 1% bacto tryptone (Difco), 1% technical casamino acids (Difco), 0.005 g of $MgSO_4$, 0.01% $FeSO_4$, 0.12% sodium citrate, 0.02% KCl, 0.04% K_2HPO_4,

0.06% L-cysteine, and 1.5% glucose at 37°C (for details, see **Supplemental Tabel 26**) (Chen et al., 2017).

2.2.1.1.2 ^{13}C-labelling Experiments of *Francisella* Strains

1 L of growth medium (medium T) was supplemented with 2 g of [U-$^{13}C_6$]glucose (11 mM), 2 g of [1,2-$^{13}C_2$]glucose (11 mM), 0.3 g of [U-$^{13}C_3$]Ser (3 mM), or 2.5 g of [U-$^{13}C_3$]glycerol (25 mM), respectively. Volumes of 250 ml of supplemented medium T were inoculated with 2–4 ml of an over-night culture of the *Francisella* strains. Incubation was carried out at 37°C and 220 rpm. The optical density at 600 nm (OD600) was determined at regular intervals. An OD600 of ~1.8 correlated with stationary growth. Cultures in medium T reached stationary growth at 26 h. Before harvesting, a culture aliquot was plated onto lysogeny broth (LB) agar (Bertani, 1951, 2004) to rule out the possibility of contamination. The bacteria were pelleted at 4,700 g and 4°C for 15 min. The supernatant was discarded and the bacterial pellet was autoclaved at 120°C for 20 min. Then, the pellet was resuspended in 3 ml of water and lyophilized (Chen et al., 2017).

2.2.1.1.3 ^{2}H-Labelling experiments of *Francisella* strains

1 L of growth medium (CDM) was supplemented with (i) 1.7 g myo-inositol-C-d6 (9.13 mM) and 3.9 g myo-inositol (21.65 mM). For labelling experiments in CDM, bacteria were cultivated in medium T overnight. On the next day, bacterial cultures were washed two times and grown in 200 ml supplemented CDM at 37°C and 220 rpm (starting at OD600 of 0.3) to an OD600 of maximum. The optical density at 600 nm (OD600) was determined at regular intervals. An OD600 of ~0.8-1.0 correlated with stationary growth. Cultures in myo-inositol supplemented CDM reached stationary growth at ~50 h. Before harvesting, a culture aliquot was plated onto lysogeny broth (LB) agar (Bertani, 1951; 2004) to rule out the possibility of contamination. The bacteria were pelleted at 4,700 g and 4°C for 15 min. The supernatant was discarded, washed once with 200 ml cold PBS and the bacterial pellet was autoclaved at 120°C for 20 min. Then, the pellet was resuspended in 3 ml of water and lyophilized.

2.2.1.2 Analysis of polar metabolites from bacterial extraction

In a 2 mL plastic tube, 10 mg of bacterial cells (dry weight) were mixed with 500 µL of glass beads (0.25–0.5 mm) and 1 mL of methanol p.a. The mixture was mechanically disrupted using a ribolyser system (6.5 s^{-1}, 20 s, 27°C, three times). After the procedure, the mixture was centrifuged (10,000 g for 20 min, 4°C). The supernatant was collected into a 2 mL glass bottle. The solvent was removed by

a gas flow of nitrogen. 50 µL of N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide containing 1% tert-butyldimethylsilylchloride and 50 µL of water-free acetonitrile were added and incubated at 70°C for 1 h. The reaction mixture was collected and subjected to GC-MS analysis.

2.2.1.3 Analysis of free fructose from bacterial extraction

In a 2 mL plastic tube, about 10 mg of bacterial sample (dry weight) were mixed with about 500 µL of glass beads (0.25–0.5 mm) and 1 mL of water. The bacterial cells were mechanically disrupted using a ribolyser system (6.5 s^{-1}, 20 s, 27°C, three times). After the procedure, the mixture was centrifuged (10,000 g for 20 min, 4°C) and the supernatant was collected into a 2 mL glass bottle. The solvent was removed by a gas flow of nitrogen. 100 µL of pyridine containing 4 mg of methoxamine hydrochloride were added and incubated at 30°C for 90 minutes. The solvent was then removed by a gas flow of nitrogen. 100 µL of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide were added, kept at 37°C for 45 minutes, and then subjected to GC-MS analysis.

2.2.1.4 Analysis of total amino acids from bacterial hydrolysate

In a 2 mL glass bottle about 2 mg of bacterial sample was weighed, 0.5 mL of 6 M hydrochloric acid was added, the mixture was incubated at 105°C for 15 hours and then filtered. The hydrolysate was dried by a gas flow of nitrogen. 200 µL of 50% acetic acid were added. After 3 min, the mixture was treated on an ultrasonic bath and then loaded onto a small column of Dowex 50W X8 (7 × 10 mm; 200–400 mesh, 34–74 µm, H+-form). After washing with 2 mL of water, the amino acids were eluted with 1 mL of 4 M ammonia water into a 2 mL glass bottle. Under nitrogen flux, the solvent was removed. 50 µL of N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide containing 1% tert-butyldimethylsilylchloride and 50 µL of water-free acetonitrile were added and incubated at 70°C for 30 min. The reaction mixture was collected and subjected to GC-MS analysis. During the hydrolysis, Cys and Trp were degraded and could therefore not be detected. Asn and Gln were converted into Asp and Glu, respectively, by the acidic treatment. The values reported below for Asp and Glu represent the mean value for Glu/Gln and Asp/Asn, respectively.

2.2.1.5 Analysis of glucose from bacterial polysaccharides hydrolysate

In a 2 mL glass bottle, about 0.5 mg of bacterial cells (dry weight) were mixed with 0.5 mL of 3 M methanolic HCl. The mixture was incubated at 80°C for 15 h. After cooling, the mixture was transferred into a 2 mL plastic tube and then centrifuged (10,000 g for 20 min, 4°C). The supernatant

was collected into a 2 mL glass bottle and dried under a flow of nitrogen. 1 mL of acetone containing 2% sulfuric acid was added. The mixture was incubated at room temperature for 1 h. 2 mL of saturated sodium chloride solution and 2 mL of saturated sodium carbonate solution were added to quench the reaction. The solution was extracted two times with 3 mL of ethyl acetate and collected into a 2 mL glass bottle. The ethyl acetate solvent was dried under a flow of nitrogen and the residue was treated with 200 µL of 50% acetyl anhydride in water-free ethyl acetate at 60°C for 15 h. The reaction mixture was dried under a flow of nitrogen and 100 µL of water-free ethyl acetate were added. The solution was collected and subjected to GC-MS analysis.

2.2.1.6 Analysis of amino sugars from bacterial cell wall hydrolysate

In a 2 mL glass bottle about 15 mg of cell sample was weighed, 0.5 mL of 6 M hydrochloric acid was added, the glass bottle was incubated at 105°C for 15 hours. The mixture was then filtered and the hydrolysate was dried under a flow of nitrogen gas. Catalytic amounts of ammonium sulfate and 100 µL of hexamethyldisilazane were added and kept at 120°C for 3 h. The reaction mixture was collected and subjected to GC-MS analysis.

2.2.2 *Francisella* medium samples

2.2.2.1 Analysis of polar metabolites from medium T

In a 2 mL plastic tube 1 mL medium was added, the medium was dried under nitrogen gas. It was then mixed with 500 µL of glass beads (0.25–0.5 mm) and 1 mL of methanol p.a. The mixture was mechanically disrupted using a ribolyser system (6.5 s^{-1}, 20 s, 27°C, three times). After the procedure, the mixture was centrifuged (10,000 g for 20 min, 4°C). The supernatant was collected into a 2 mL glass bottle. The solvent was removed by a gas flow of nitrogen. 5 µL of 10 mM norvaline water solution was added to the residue, the residue was dried again. 50 µL of N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide containing 1% tert-butyldimethylsilylchloride and 50 µL of water-free acetonitrile were added and incubated at 70°C for 1 h. The reaction mixture was collected and subjected to GC-MS analysis.

2.2.2.2 Analysis of total amino acids from medium T hydrolysate

In a 2 mL glass bottle, 50 µL of medium sample was added, then the medium was dried under nitrogen gas. 0.5 mL of 6 M hydrochloric acid was added, the glass bottle was incubated at 105°C for 15 hours.

The hydrolysate was then desiccated by blowing with nitrogen gas, 200 µL of 50% acetic acid was added after 3 minutes treatment on an ultrasonic bath, the mixture was loaded onto a small column of Dowex 50W X8 (7 × 10 mm; 200–400 mesh, 34–74 µm, H+-form). After washing with 2 mL of water, the amino acids were eluted with 1 mL of 4 M ammonia water into 2 mL glass bottle. Under nitrogen flux the ammonia water was volatilized, 5µL of 10 mM norvaline water solution was added, it was dried again under N2. 50 µL MTBSTFA (with 1% t-BDMCS) and 50 µL water free acetonitrile were added into the glass bottle, then the mixture was incubated at 70°C for 30 minutes. The reactant was collected and subjected to GC-MS analysis.

2.2.2.3 Analysis of free glucose from medium T

In a 2 mL glass bottle, 1 mL of medium was added, it was dried under nitrogen gas. 100µL of 10 mM fructose water solution was added, it was dried again. It were then mixed with 0.5 mL of 3 M methanolic HCl. The mixture was incubated at 80°C for 15 h. After cooling, the mixture was transferred into a 2 mL plastic tube and then centrifuged (10,000 g for 20 min, 4°C). The supernatant was collected into a 2 mL glass bottle and dried under a flow of nitrogen. 1 mL of acetone containing 2% sulfuric acid was added. The mixture was incubated at room temperature for 1 h. 2 mL of saturated sodium chloride solution and 2 mL of saturated sodium carbonate solution were added to quench the reaction. The solution was extracted two times with 3 mL of ethyl acetate and collected into a 2 mL glass bottle. The ethyl acetate solvent was dried under a flow of nitrogen and the residue was treated with 200 µL of 50% acetyl anhydride in water-free ethyl acetate at 60°C for 15 h. The reaction mixture was dried under a flow of nitrogen and 100 µL of water-free ethyl acetate were added. The solution was collected and subjected to GC-MS analysis.

2.2.3 *Bacillus subtilis* experiments

2.2.1.1 *Bacillus subtilis* cultivation

These cultivation experiments were done by Jan Kampf at the University of Göttingen under the supervision of Prof. Dr. Jörg Stülke.

2.2.1.1.1 Mutant, Growth Conditions, Media and Buffers

Three *B. subtilis* mutant were used in this work. They are derived from the non-domesticated wild type strain NCIB3610. *B. subtilis* was typically grown in LB medium or in Spizizen minimal medium

containing glucose and glutamate as sources of carbon and nitrogen, respectively (Commichau et al., 2008). Alternatively, SP medium (8 g/ml nutrient broth, 1 mM $MgSO_4$, and 13 mM KCl supplemented after sterilization with 2.5 µM ferric ammonium citrate, 500 µM CaCl2, and 10 µM MnCl2) (Amory et al., 1987), CSE glucose medium (for details, see **Supplemental Tabel 72**) (Commichau et al., 2008), YT medium (8 g/l tryptone, 5 g/l yeast extract, 2.5 g/l NaCl, pH 7.0), or MSgg medium (biofilm promoting minimal medium; for details, see Supplemental Tabel 74) (Branda et al., 2001) was used. Plates were prepared by the addition of 17 g Bacto agar/l (Difco) to the medium. To transfer mutations into the background of the non-domesticated wild-type strain NCIB3610, SPP1-mediated phage transduction was used as described previously (Diethmaier et al., 2011). Transductants were selected on CSE glucose and YT plates containing spectinomycin at a concentration of 150 µg/ml.

2.2.1.1.2 ^{13}C-Labelling experiments of *B. subtilis*

2.2.1.1.2.1 Labelling experiments in liquid culture using CSE glucose medium containing [U-$^{13}C_6$]glucose

B. subtilis cells were cultured in 4 ml LB medium at 37°C and 200 rpm. 10 µl of the pre-culture were used to inoculate 10 ml of CSE-glucose / ^{13}C-glucose medium and grown overnight at 28°C and 200 rpm. On the next day, 50 ml of CSE-glucose / ^{13}C-glucose cultures were inoculated to an OD_{600} of 0.05 and grown until they reached an OD_{600} of 1.0. The cultures were harvested at 8000 rpm for 10 min, washed twice with PBS pH 7.4 (137 mM NaCl, 0.2 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4) and snap frozen at -80°C.

2.2.1.1.2.2 Labelling experiments on MSgg agar containing [U-$^{13}C_6$]glucose or [U-$^{13}C_5$]glutamate

B. subtilis cells were cultured in 4 ml LB medium overnight at 37°C and 200 rpm. On the next day, 4 ml LB medium were inoculated to an OD600 of 0.1 and grown until they reached an OD600 of 0.5 - 0.9. A volume of 2 ml of the cell suspension was centrifuged for 5 min at 6000 rpm. The pellet was washed twice in MSgg medium (for details, see Supplemental Tabel 74) containing 0.5 % glucose and resuspended in 2 ml of the medium. 3 x 5 µl of the cell suspension per biological replicate were dropped on MSgg agar plates containing 0.5 % glucose. 1/3 of the glucose was D-[U-$^{13}C_6$]glucose for the labelling studies (for details, see Supplemental Tabel 74). For labelling with L-[U-$^{13}C_5$]glutamate, the cells were washed and resuspended in standard MSgg medium, and, MSgg agar plates were prepared containing 19.7 mM potassium glutamate and 5.4 mM L-[U-$^{13}C_5$]glutamate (for details, see Supplemental Tabel 74). Since it is not possible to autoclave all components of MSgg medium, single components were sterilized solely and mixed afterwards. For the preparation of plates, 1.5 % (w/v) Bacto agar for minimal medium was added to the medium. For the preparation of 500 ml

medium, deionized water was added to 7.5 g agar to a total volume of 300 ml and the mixture was autoclaved. Next, the salts and the other components (preheated) were added to the warm agar to obtain a final volume of 500 ml. To avoid precipitation of the salts, the agar was mixed continuously prior to pouring the plates. To ensure reproducible colony phenotypes, exactly 25 ml medium were used for every plate. The plates were stored in the refrigerator at 4°C (Branda et al., 2001).

These plates were used for growing the macrocolonies. Colonies were grown for three days at 30°C. The colonies were scratched off the surface, pelleted and washed twice with ice-cold PBS pH 7.4. Samples were snap frozen and stored at -80°C until they were processed (Branda et al., 2001).

2.2.3.2 Analysis of polar metabolites from bacterial extraction

In a 2 mL plastic tube, 10 mg of bacterial cells (dry weight) were mixed with 500 µL of glass beads (0.25–0.5 mm) and 1 mL of methanol p.a. The mixture was mechanically disrupted using a ribolyser system (6.5 s^{-1}, 20 s, 27°C, three times). After the procedure, the mixture was centrifuged (10,000 g for 20 min, 4°C). The supernatant was collected into a 2 mL glass bottle. The solvent was removed by a gas flow of nitrogen. 50 µL of N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide containing 1% tert-butyldimethylsilylchloride and 50 µL of water-free acetonitrile were added and incubated at 70°C for 1 h. The reaction mixture was collected and subjected to GC-MS analysis.

2.2.3.3 Analysis of protein derived amino acids from bacterial hydrolysate

After the methanol extraction, the residue was dried and collected into a 4 mL glass bottle. 2 mL of 6 M hydrochloric acid were added. The mixture was incubated at 105°C for 15 hours and then filtered. The hydrolysate was dried by a gas flow of nitrogen. 200 µL of 50% acetic acid were added. After 3 min, the mixture was treated on an ultrasonic bath and then loaded onto a small column of Dowex 50W X8 (7 × 10 mm; 200–400 mesh, 34–74 µm, H+-form). After washing with 2 mL of water, the amino acids were eluted with 1 mL of 4 M ammonia water into a 2 mL glass bottle. Under nitrogen flux, the solvent was removed. 50 µL of N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide containing 1% tert-butyldimethylsilylchloride and 50 µL of water-free acetonitrile were added and incubated at 70°C for 30 min. The reaction mixture was collected and subjected to GC-MS analysis. During the hydrolysis, Cys and Trp were degraded and could therefore not be detected. Asn and Gln were converted into Asp and Glu, respectively, by the acidic treatment. The values reported below for Asp and Glu represent the mean value for Glu/Gln and Asp/Asn, respectively.

2.2.3.4 Analysis of free fructose from bacterial extraction

In a 2 mL plastic tube, about 10 mg of bacterial sample (dry weight) were mixed with about 500 µL of glass beads (0.25–0.5 mm) and 1 mL of water. The bacterial cells were mechanically disrupted using a ribolyser system (6.5 s^{-1}, 20 s, 27°C, three times). After the procedure, the mixture was centrifuged (10,000 g for 20 min, 4°C) and the supernatant was collected into a 2 mL glass bottle. The solvent was removed by a gas flow of nitrogen. 100 µL of pyridine containing 4 mg of methoxamine hydrochloride were added and incubated at 30°C for 90 minutes. The solvent was then removed by a gas flow of nitrogen. 100 µL of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide were added, kept at 37°C for 45 minutes, and then subjected to GC-MS analysis.

2.2.3.5 Analysis of amino sugars from bacterial cell wall hydrolysate

After the water extraction, the residue was dried and collected into a 4 mL glass bottle. 2 mL of 6 M hydrochloric acid were added and the mixture was incubated at 105°C for 15 h. The mixture was then filtered and the hydrolysate was dried under a flow of nitrogen gas. Catalytic amounts of ammonium sulfate and 100 µL of hexamethyldisilazane were added and kept at 120°C for 3 h. The reaction mixture was collected and subjected to GC-MS analysis.

2.2.3.6 Analysis of glucose from bacterial polysaccharides hydrolysate

In a 2 mL glass bottle, about 0.5 mg of bacterial cells (dry weight) were mixed with 0.5 mL of 3 M methanolic HCl. The mixture was incubated at 80°C for 15 h. After cooling, the mixture was transferred into a 2 mL plastic tube and then centrifuged (10,000 g for 20 min, 4°C). The supernatant was collected into a 2 mL glass bottle and dried under a flow of nitrogen. 1 mL of acetone containing 2% sulfuric acid was added. The mixture was incubated at room temperature for 1 h. 2 mL of saturated sodium chloride solution and 2 mL of saturated sodium carbonate solution were added to quench the reaction. The solution was extracted two times with 3 mL of ethyl acetate and collected into a 2 mL glass bottle. The ethyl acetate solvent was dried under a flow of nitrogen and the residue was treated with 200 µL of 50% acetyl anhydride in water-free ethyl acetate at 60°C for 15 h. The reaction mixture was dried under a flow of nitrogen and 100 µL of water-free ethyl acetate were added. The solution was collected and subjected to GC-MS analysis.

2.2.4 Immortal brown adipose tissue

2.2.4.1 Cultivation of immortal brown adipose tissue

These cultivation experiments were done by Yongguo Li at the Technical University of Munich under the supervision of Prof. Dr. Martin Klingenspor.

Immortalized brown adipocytes were cultured in 15-cm-diameter petri dishes as previously described (Li et al., 2014 BBA). Glucose isotopic tracer experiments were performed at day 7 of adipogenic differentiation. Briefly, cells were washed once with prewarmed PBS and changed to 25mL seahorse respiration medium [DMEM basal medium (Sigma-D5030) supplemented with 25 mM glucose, 31 mM NaCl, 2 mM GlutaMax and 15 mg/l phenol red, pH 7.4] to mimic the seahorse bioenergetic profiling procedure. After 1h preincubation at 37°C in a room air incubator, cells were treated with oligomycin (5 µM) to block the coupled respiration for 30 min. Then medium was changed to the glucose isotopic tracer medium [DMEM basal medium (Sigma-D5030), for details, see Supplemental Table 89] supplemented with 20 mM glucose, 5mM [U-$^{13}C_6$]glucose, 31 mM NaCl, 2 mM GlutaMax and 15 mg/l phenol red, pH 7.4)) with oligomycin (5 µM) and treated with or without isoproterenol (0.5 µM) for 30 min. Before harvesting, cells were washed once with cold PBS, 15 ml methanol was added and stored at –80˚C until further analysis.

2.2.4.2 Analysis of polar metabolites from cell extraction

In a 2 mL plastic tube, about 20 mg of cells (dry weight) were mixed with 500 µL of glass beads (0.25–0.5 mm) and 1 mL of extractant (methanol:chloroform:water = 5:2:1). The mixture was mechanically disrupted using a ribolyser system (6.5 s^{-1}, 20 s, 27°C, three times). After the procedure, the mixture was centrifuged (10,000 g for 20 min, 4°C). The supernatant was collected into a 2 mL glass bottle. The solvent was removed by a gas flow of nitrogen. 50 µL of 100 mM norvaline water solution was added to the residue, the residue was dried again. 50 µL of N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide containing 1% tert-butyldimethylsilylchloride and 50 µL of water-free acetonitrile were added and incubated at 70°C for 1 h. The reaction mixture was collected and subjected to GC-MS analysis.

2.2.4.3 Analysis of total amino acids from cell hydrolysate

In a 2 mL glass bottle about 1 mg of cells (dry weight) were mixed with 0.5 mL of 6 M hydrochloric

acid was added. The mixture was incubated at 105°C for 15 hours and then filtered. The hydrolysate was dried by a gas flow of nitrogen. 200 µL of 50% acetic acid were added. After 3 min, the mixture was treated on an ultrasonic bath and then loaded onto a small column of Dowex 50W X8 (7 × 10 mm; 200–400 mesh, 34–74 µm, H+-form). After washing with 2 mL of water, the amino acids were eluted with 1 mL of 4 M ammonia water into a 2 mL glass bottle. Under nitrogen flux, the solvent was removed. 50 µL of N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide containing 1% tert-butyldimethylsilylchloride and 50 µL of water-free acetonitrile were added and incubated at 70°C for 30 min. The reaction mixture was collected and subjected to GC-MS analysis. During the hydrolysis, Cys and Trp were degraded and could therefore not be detected. Asn and Gln were converted into Asp and Glu, respectively, by the acidic treatment. The values reported below for Asp and Glu represent the mean value for Glu/Gln and Asp/Asn, respectively.

2.2.4.4 Analysis of glucose from cell polysaccharides hydrolysate

In a 2 mL glass bottle, about 5 mg of cells (dry weight) were mixed with 0.5 mL of 3 M methanolic HCl. The mixture was incubated at 80°C for 15 h. After cooling, the mixture was transferred into a 2 mL plastic tube and then centrifuged (10,000 g for 20 min, 4°C). The supernatant was collected into a 2 mL glass bottle and dried under a flow of nitrogen. 1 mL of acetone containing 2% sulfuric acid was added. The mixture was incubated at room temperature for 1 h. 2 mL of saturated sodium chloride solution and 2 mL of saturated sodium carbonate solution were added to quench the reaction. The solution was extracted two times with 3 mL of ethyl acetate and collected into a 2 mL glass bottle. The ethyl acetate solvent was dried under a flow of nitrogen and the residue was treated with 200 µL of 50% acetyl anhydride in water-free ethyl acetate at 60°C for 15 h. The reaction mixture was dried under a flow of nitrogen and 100 µL of water-free ethyl acetate were added. The solution was collected and subjected to GC-MS analysis.

2.2.4.5 Analysis of fatty acid from cell lipid hydrolysate

In a 2 mL glass bottle, about 5 mg of cells (dry weight) were mixed with 0.5 mL of 3 M methanolic HCl. The mixture was incubated at 80°C for 15 h. After cooling, the mixture was transferred into a 2 mL plastic tube and then centrifuged (10,000 g for 20 min, 4°C). The supernatant was collected into a 2 mL glass bottle and dried under a flow of nitrogen. The residue was resolved in 100 µL water free hexane and subjected to GC-MS analysis.

2.2.5 Analysis of DMEM medium

2.2.5.1 Analysis of polar metabolites from DMEM medium

In a 2 mL plastic tube, 1 mL DMEM medium was added, the medium was dried under nitrogen gas. It was then mixed with 500 µL of glass beads (0.25–0.5 mm) and 1 mL of extractant (methanol:chloroform:water = 5:2:1). The mixture was mechanically disrupted using a ribolyser system (6.5 s^{-1}, 20 s, 27°C, three times). After the procedure, the mixture was centrifuged (10,000 g for 20 min, 4°C). The supernatant was collected into a 2 mL glass bottle. The solvent was removed by a gas flow of nitrogen. 50 µL of 100 mM norvaline water solution was added to the residue, the residue was dried again. 50 µL of N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide containing 1% tert-butyldimethylsilylchloride and 50 µL of water-free acetonitrile were added and incubated at 70°C for 1 h. The reaction mixture was collected and subjected to GC-MS analysis.

2.2.5.2 Analysis of free glucose from medium

In a 2 mL glass bottle, 1 mL medium sample was added, then the medium was dried under nitrogen gas. It was then mixed with 0.5 mL of 3 M methanolic HCl. The mixture was incubated at 80°C for 15 h. After cooling, the mixture was transferred into a 2 mL plastic tube and then centrifuged (10,000 g for 20 min, 4°C). The supernatant was collected into a 2 mL glass bottle and dried under a flow of nitrogen. 1 mL of acetone containing 2% sulfuric acid was added. The mixture was incubated at room temperature for 1 h. 2 mL of saturated sodium chloride solution and 2 mL of saturated sodium carbonate solution were added to quench the reaction. The solution was extracted two times with 3 mL of ethyl acetate and collected into a 2 mL glass bottle. The ethyl acetate solvent was dried under a flow of nitrogen and the residue was treated with 200 µL of 50% acetyl anhydride in water-free ethyl acetate at 60°C for 15 h. The reaction mixture was dried under a flow of nitrogen and 100 µL of water-free ethyl acetate were added. The solution was collected and subjected to GC-MS analysis.

2.2.6 GC/MS analysis

All derivatives mentioned above were analysed by GC-MS using a quadrupol GCMS-QP 2010 Plus spectrometer (Shimadzu, Duisburg, Germany) as described earlier (Chen et al., 2017). The GC column was a Silica capillary column (equity TM-5; 30 m by 0.25 mm, 0.25-µm film thickness; Sigma-Aldrich). For the analysis of silylated fructose and other polar metabolites, the column was developed at 100°C for 2 min, then at a gradient up to to 234°C (3°C per min), later at a gradient up to 237°C (1°C per min), and finally at a gradient up to 260°C (3°C per min). At the end, the column was quickly heated to 320°C (10°C per min) and held at this temperature for 2 min. For the analysis of

silylated amino acids, the column was developed at 150°C for 3 min, then at a gradient up to 280°C (7°C per min) which was hold for 3 min. For the analysis of the diisopropylidene/acetate derivative of glucose, the column was developed at 150°C for 3 min, then at a gradient up to 220°C (10°C per min), followed at a gradient up to 280°C (50°C per min) which was hold for 3 min. For the analysis of silylated amino sugars, the column was developed at 70°C for 3 min, then at a gradient up to 310°C (5°C per min) which was hold for 1 min.

All data were collected using the LabSolution software (Shimadzu). Each sample was analyzed three times to afford technical replicates. The overall ^{13}C excess values (mol-%) and the relative contributions of isotopologues (%) were computed by an Excel-based in-house software package (Eylert et al., 2008) according to Lee et al. (Lee et al., 1991). Alternatively, $^{2}H/^{13}C$ excess and isotopologue compositions were calculated according to Ahmed et al. (2014). This software package is open source and can be downloaded using the following link: http://www.tr34.uni-wuerzburg.de/software_developments/isotopo/.

Retention times and mass fragments that were used for calculations of overall $^{2}H/^{13}C$-exces values and isotopologue composition are shown in **Supplemental Table 27**.

3. Results and discussion

3.1 Differential substrate usage and metabolic fluxes in *Francisella tularensis* subspecies *holarctica* and *Francisella novicida*

3.1.1 Experimental approach

To find out the differential usages of substrates for the growth of different *Francisella* strains, we performed labelling experiments using the stable ^{13}C-precursors, [U-$^{13}C_6$]glucose, [1,2-$^{13}C_2$]glucose, [U-$^{13}C_3$]serine or [U-$^{13}C_3$]glycerol supplemented to medium T. Three different *Francisella* strains were studied in this work: a highly pathogenic *Francisella tularensis* subspecies *holarctica* WT strain which was isolated from beaver (Schulze et al., 2016), the *Francisella tularensis* subspecies *holarctica* life vaccine strain LVS and the less pathogenic *Francisella novicida* strain U112. Each of these strains was grown in medium T supplemented with 11 mM [U-$^{13}C_6$]glucose, 11 mM [1,2-$^{13}C_2$]glucose, 3 mM [U-$^{13}C_3$]serine or 25 mM [U-$^{13}C_3$]glycerol at 37 ^{o}C for 26 h. The cells were harvested at an OD600 value of approximately 1.8. Then the cells were washed twice with PBS solution, centrifuged, pelleted, autoclaved and lyophilized to get about 100 mg of dried cell pellet. Using physical or chemical procedures different sugars from the cell wall or from glycogen, protein derived or free amino acids and free polar metabolites were isolated from the dried cell pellets. All of these components were then treated with different derivatisation reagents to convert them into a non-polar volatile form which could be analysed by GC/MS spectrometry (for details, see Materials and Methods). The labelling experiments were repeated at least once (biological replicates), and each sample was analysed three times by GC/MS spectrometry (technical replicates). Based on the biological replicates and technical replicates, at least six experimental values of each metabolite were detected. Mean values and standard deviations of each metabolite were then calculated on this basis (see **Supplemental Tables S1-27** for numerical data).

3.1.2 Labelling experiments with [U-$^{13}C_6$]glucose

3.1.2.1 Isotopolog profiles of sugars

From the dried cell pellets, four different sugars were isolated namely (i) free fructose, which was isolated from the aqueous cell extracts (ii) glycogen derived glucose, which was a hydrolysis product of the cell pellet treating with 3 M methanolic hydrochloric acid and (iii) cell wall derived amino sugars glucosamine and muramic acid, which were obtained by harsh 6 M hydrochloric acid treatment. Each sugar was highly labelled with ^{13}C-enrichments of 10-12%, which reflected the efficient uptake and usage of glucose. In the *Francisella tularensis* subspecies *holarctica* WT (*Fth*) the four hexoses

were slightly higher labelled (11.2 ± 0.9%) than those in *Francisella novicida* strain U112 (*Fno*) (10.5 ± 1.1%) (See also **Supplemental Tables S9**). Free fructose was mainly M+6 labelled, which reflects its origin from [U-$^{13}C_6$]glucose via glucose 6-phosphate and fructose 6-phosphate. Surprisingly, the isotopolog composition in different sugars were highly different, the M+1 to M+5 isotopolog fractions in free fructose was 20-30%, in glycogen derived glucose 20-50% and in cell wall derived amino sugars glucosamine and muramic acid 60-90% (**Figure 9B**), which suggests more frequent metabolic turnover at higher rates during the formation of cell wall derived amino sugars. The fractional isotopolog distributions of different sugars in *Francisella tularensis* subspecies *holarctica* WT (*Fth*) and *Francisella novicida* strain U112 (*Fno*) were apparently similar, however, the glycogen derived glucose in *Francisella novicida* strain U112 (*Fno*) had significant lower ^{13}C-enrichments than that in *Francisella tularensis* subspecies *holarctica* WT (*Fth*).

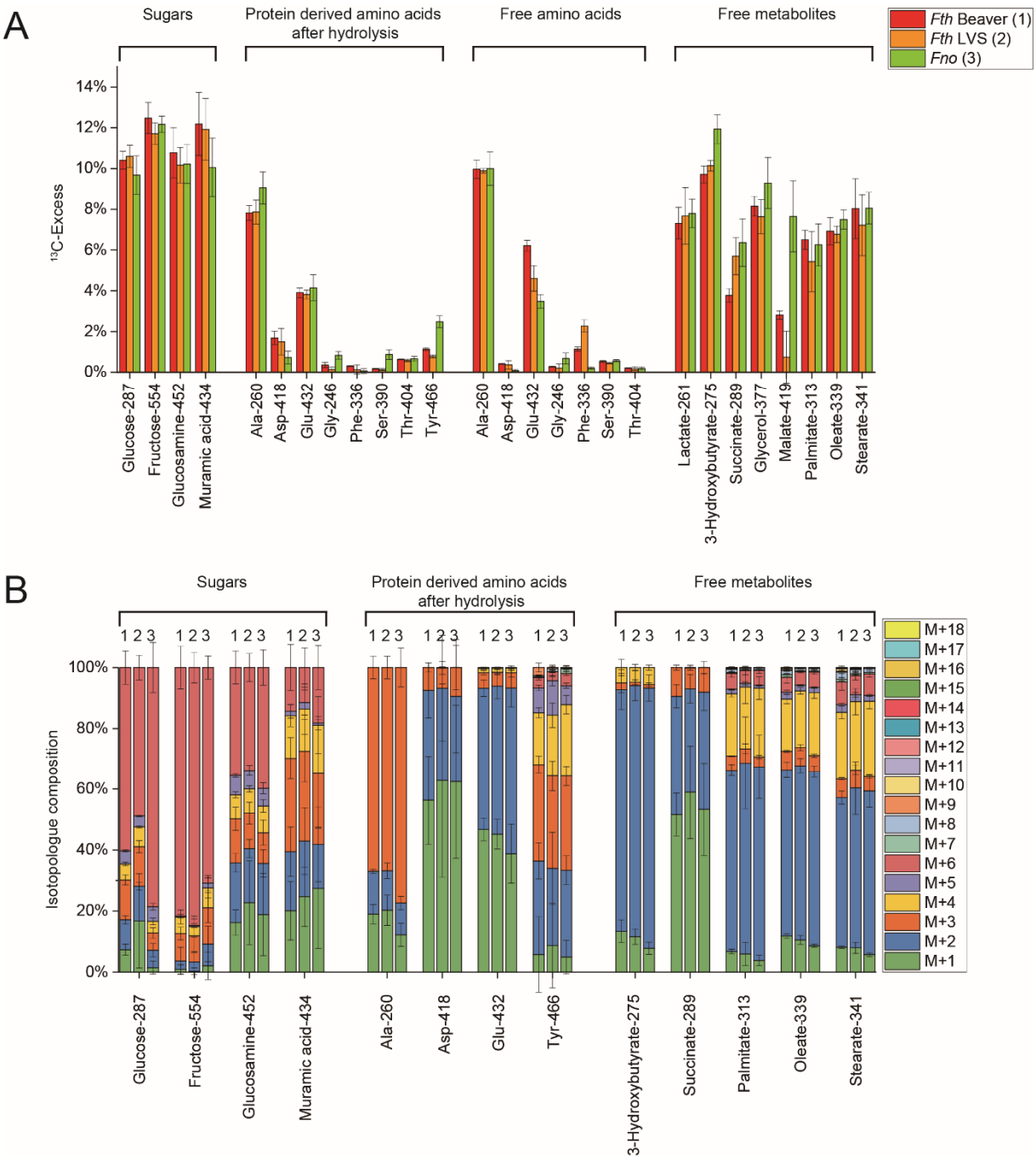


Figure 9. (A) ^{13}C-Excess (mol%) and (B) the fractional isotopolog distributions (%) in key metabolites of *Francisella tularensis* subspecies *holartica* WT strain (beaver isolate) (*Fth* Beaver, 1), the *Francisella tularensis* subspecies *holartica* life vaccine strain LVS (*Fth* LVS, 2), or the less pathogenic *Francisella novicida* strain U112 (*Fno*, 3) grown in medium T supplied with 11 mM [U-$^{13}C_6$]glucose. ^{13}C-Excess (mol%) and relative fractions of isotopologs (%) were determined by GC/MS of silylated derivatives at the indexed m/z values. Error bars indicate standard deviations from the means of 6 to 9 values (i.e. 2–3 × biological replicates, 3 × technical replicates). M+1, M+2, M+3, etc. indicate isotopologs carrying 1, 2, 3, etc. ^{13}C-atoms. For numerical values, see **Supplemental Tables S1**, **S5**, **S9**, **S13**, **S17**, **S21** (Chen et al., 2017).

3.1.2.2 Isotopolog profiles of protein derived amino acids

Using 6 M hydrochloric acid, the bacterial pellet was hydrolysed and the resulting mixture of amino acids, mostly derived from the protein fraction, were converted into TBDMS-derivatives which were analysed by GC/MS. During the hydrolysis, Cys and Trp were destroyed and could not be detected; because of the deamidation Asn and Gln were converted into Asp and Glu. GC/MS analysis of 15 TBDMS-amino acids revealed in *Fth* strains ^{13}C enrichments (and therefore signalling de novo biosynthesis from the labelled tracer) in 7 amino acids: Ala > Glu > Asp > Tyr > Thr > Phe > Gly = Ser (8–0.1%) and in *Fno*: Ala > Glu > Tyr >> Thr = Asp = Gly = Ser > Phe) (9–0.1%) (**Figure 9A**). Because of the unlabelled substrates (brain heart infusion broth, bacto trypton and casamino acids) in medium T, His, Ile, Leu, Val, Lys, and Pro were unlabelled due to efficient import of these amino acids from the medium into the cells and direct usage for protein biosynthesis (see also **Supplemental Tables S25**, **S26**).

No significant differences were found in the ^{13}C-excess (mol%) values (**Figure 9A**) and the fractional isotopolog distributions (**Figure 9B**) between the highly pathogenic *Fth* WT strain, the *Fth* life vaccine strain LVS. However, comparing *Fno* with the *Fth* strains, significant differences could be found. The amino acids (especially Ala, Ser, and Tyr) which derived from protein, had much higher ^{13}C-incorpration in *Fno* than in *Fth* strains. Also the obtained isotopolog distributions displayed differences between *Fth* strains and *Fno*.

Alanine, which was degraded from [U-$^{13}C_6$]glucose via [U-$^{13}C_3$]pyruvate, was mainly M+3 labelled indicating efficient uptake and glycolytic usage of glucose from medium T. Sequence data of *Francisella* strains show that the enzymes for glycolysis (EMP pathway) and non-oxidative PPP are present in the genomes (**Figure 2**). However, in the *Francisella* strains' genomes, alanine dehydrogenase and Glu/Asp transaminase converting [U-$^{13}C_3$]pyruvate into [U-$^{13}C_3$]alanine are not annotated. The M+1 and M+2 labelled alanine could indicate their origin from M+1 and M+2 in Asp by an aspartate 4-decarboxylase, which in turn is reflected in the genomes. *Fth* strains were 65% M+3 labelled and *Fno* was 75% M+3 labelled indicating that 65% of alanine in *Fth* strains were formed from pyruvate and that 75% of alanine in *Fno* were formed from pyruvate (**Figure 9B**). The ^{13}C-flux entered the TCA cycle from labelled pyruvate via [U-$^{13}C_2$]acetyl-CoA resulting in the formation of $^{13}C_2$-Glu and $^{13}C_2$-Asp from $^{13}C_2$-α-ketoglutarate and $^{13}C_2$-oxaloacetate, respectively. The isotopolog distribution of Tyr was more complex since Tyr was formed from erythrose 4-phosphate and PEP via shikimate/chorismate pathway. [U-$^{13}C_4$]erythrose 4-phosphate can be formed via the PPP from [U-$^{13}C_6$]fructose 6-phosphate by transketolase, resulting in a large M+4 fraction in Tyr. During the incorporation of [U-$^{13}C_3$]PEP, one of the PEP splits off a ^{13}C atom, resulting in the observed M+2 and

M+3 fractions in Tyr.

3.1.2.3 Isotopolog profiles of free polar metabolites

Using methanol as an extractant, more than 20 polar metabolites including free amino acids, lactate, glycerol, succinate, malate and free fatty acids were isolated from the dried bacterial pellets. Generally, the ^{13}C-excess values and isotope distributions of free amino acids were similar to those in protein derived amino acids (**Figure 9A**). Lactate and glycerol had similar ^{13}C-profiles as alanine suggesting that the related metabolites were in a quasi-equilibrium state of isotope distribution.

Free fatty acids and 3-hydroxybutyrate were highly labelled (6-12%) in form of M+2 isotopologs indicating the efficient usage of [U-$^{13}C_6$]glucose for fatty acid formation via [U-$^{13}C_2$]acetyl-CoA. [U-$^{13}C_2$]acetyl-CoA is also used in the TCA forming succinate and malate as M+2 species. The polar metabolites in *Fno* and *Fth* strains had similar ^{13}C-excess and fractional isotopolog distributions, with a slight tendency of higher incorporation into metabolites in *Fno* as compared to *Fth* strains (**Figure 9**).

In total, from the ^{13}C patterns of different metabolites from the [U-$^{13}C_6$]glucose labelling experiment, glucose was suggested (i) as an efficient precursor to be metabolized into glycogen, and (ii) as a substrate for degradation via the glycolytic pathway to form pyruvate/Ala and acetyl-CoA (lower rates) serving as a precursor for fatty acids and driving the TCA intermediates biosynthesis. However, the complex isotope distributions in cell wall amino sugars (glucosamine and muramic acid) also suggested significant rates of glycolytic cycling via the PPP and/or gluconeogenesis. In comparison of *Fth* strains and *Fno*, glucose was used more efficiently for the biosynthesis of cell wall amino sugars in *Fth* strains, whereas in *Fno* the degradation and downstream glucose usage was more pronounced.

3.1.3 Labelling experiments with [1,2-$^{13}C_2$]glucose

To determine whether glucose is degraded via glycolysis, the PPP or the Entner-Doudoroff pathway, we performed additional experiments with [1,2-$^{13}C_2$]glucose as a tracer. In this experiment, the key fragment of the TBDMS derivative of alanine (Ala-232) which is devoid of C-1(**Figure 10A**) was used to discriminate between the pathways. Comparing to the mass fragment of alanine (Ala-260), which carried the three carbon atoms of the original Ala, some positional ^{13}C-assignment can be made. Ala-260 had high M+2 fraction, because of the [1,2-$^{13}C_2$]glucose labelling. The Ala-232 had similar high M+2 fractions as in Ala-260. This indicated that the C-1 of alanine was not ^{13}C labelled (**Figure**

10A). Consequently, the ^{13}C atoms of [1,2-$^{13}C_2$]glucose were transferred into C-2 and C-3 of alanine reflecting the Embden-Meyerhof-Parnsa (EMP) pathway. The conversion of [1,2-$^{13}C_2$]glucose into [1,2-$^{13}C_2$]pyruvate and [1,2-$^{13}C_2$]alanine via the Entner-Doudoroff pathway would result in different labelling patterns (**Figure 10B**). The degradation of [1,2-$^{13}C_2$]glucose via the PPP would result in only single labelled or unlabelled pyruvate/Ala. Therefore, the PPP pathway could also be excluded as the major degradation pathway of glucose. Thus, the EMP pathway is the predominant route for glucose degradation.

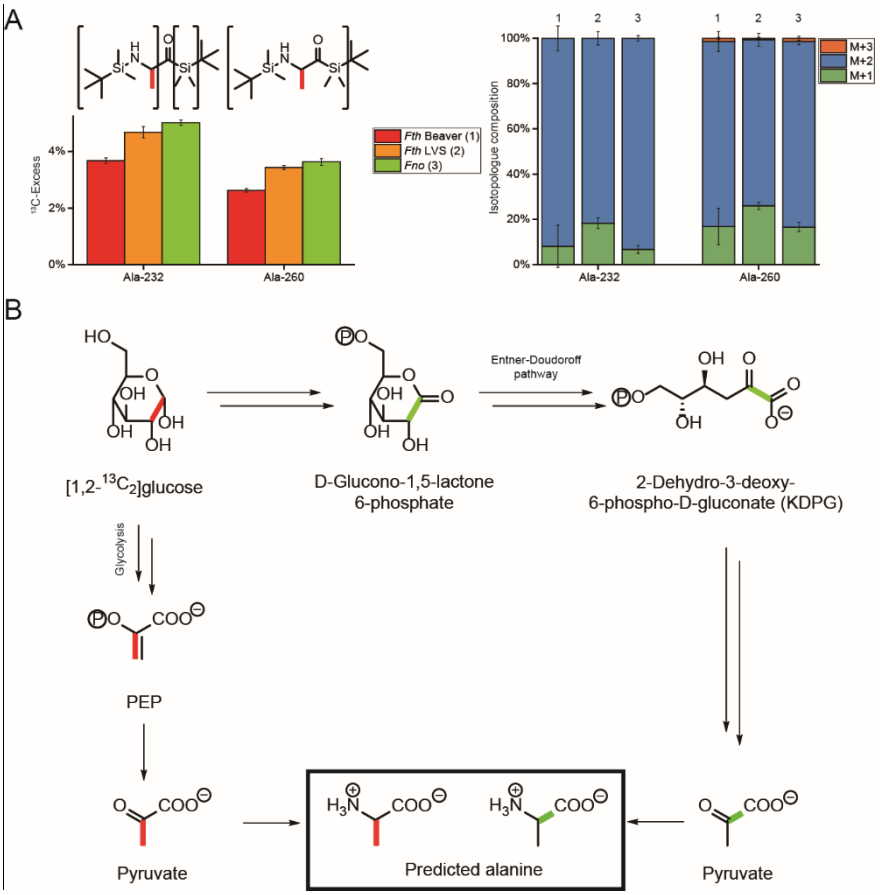


Figure 10. Transfer of ^{13}C-label from [1,2-$^{13}C_2$]glucose into alanine of *Fth* Beaver (1), *Fth* LVS (2), or *Fno* (3). (A) ^{13}C-Excess (mol%) and the fractional isotopolog distributions (%) in the silylated fragments Ala-260 and Ala-232 carrying C_1-C_3 and C_2-C_3 of the original alanine carbon chain, respectively. (B) Conversion of [1,2-$^{13}C_2$]glucose into pyruvate and alanine via glycolysis (EMP) (left, with red bars indicating the ^{13}C-labels) or the Entner-Doudoroff pathway (ED) (right, with green bars indicating the ^{13}C-labels). The observed label distribution with ^{13}C at C_2 and C_3 (indicated by red bars in panel A), only matches the predicted pattern via the EMP in panel (B). For more details, see also text (Chen et al., 2017).

3.1.3 Labelling experiments with [U-$^{13}C_3$]serine

Figure 11 shows the ^{13}C-enrichments and isotopolog distributions of obtained metabolites from the labelling experiment with [U-$^{13}C_3$]serine. The ^{13}C-enrichment of methanol-extracted free serine (*Fth* beaver isolate, 10%; *Fth* LVS, 6%, *Fno*, 20%) reflects immediately the uptake of [U-$^{13}C_3$]serine (See

also **Supplemental Table S7**, **Figure 11A**). In addition, the ^{13}C-enrichment of protein derived Ser was 12% and 16% in the *Fth* strains and in the *Fno* strain, repectively. Free serine as well as protein derived serine displayed mainly M+3 labelling suggesting less metabolic turnover leading to M+1 or M+2 serine (**Figure 11B**).

However, ^{13}C-serine could be metabolised as shown by labelled glycine, which could be formed from serine by a hydroxymethytransferase, leading to M+2. Serine was also degraded to form pyruvate, which later formed alanine and was transferred into the TCA. Therefore, Ala, lactate (in *Fno* strain), Asp, Glu (from the TCA) and free fatty acids (derived from acetyl-CoA) and 3-hydroxybutyrate (derived from acetyl-CoA) were significantly labelled. However, serine was not effectively used for gluconeogenesis, as indicated by the apparent lack of ^{13}C-incorpration into amino acids from the PPP (Phe and Tyr) and the sugars (glucose from glycogen, free fructose and amino sugar from cell wall) (**Supplemental Table S3**, **S7**, **S11**).

Fno and *Fth* strains showed different ^{13}C-profies. In *Fth* strains, serine was used more efficiently to form amino acids and proteins (with the exception of glycine) via pyruvate and oxaloacetate, and fatty acids via acetyl-CoA. However, in *Fno*, the free amino acids Ala, Gly and Ser, as well as pyruvate derived lactate were much higher labelled (> factor 3). Interestingly, the flux from Ser into Gly in *Fth* strains was much lower than the flux from Ser to Ala, which in *Fno* strain was *vice versa* (**Figure 11A**). It can be speculated that during the exponential phase of *Fth* strains, serine was more effectively used, whereas during the post-exponential phase or stationary phase it was less used as indicated by the lower ^{13}C-enrichment in the free metabolites when harvested during the late growth phase.

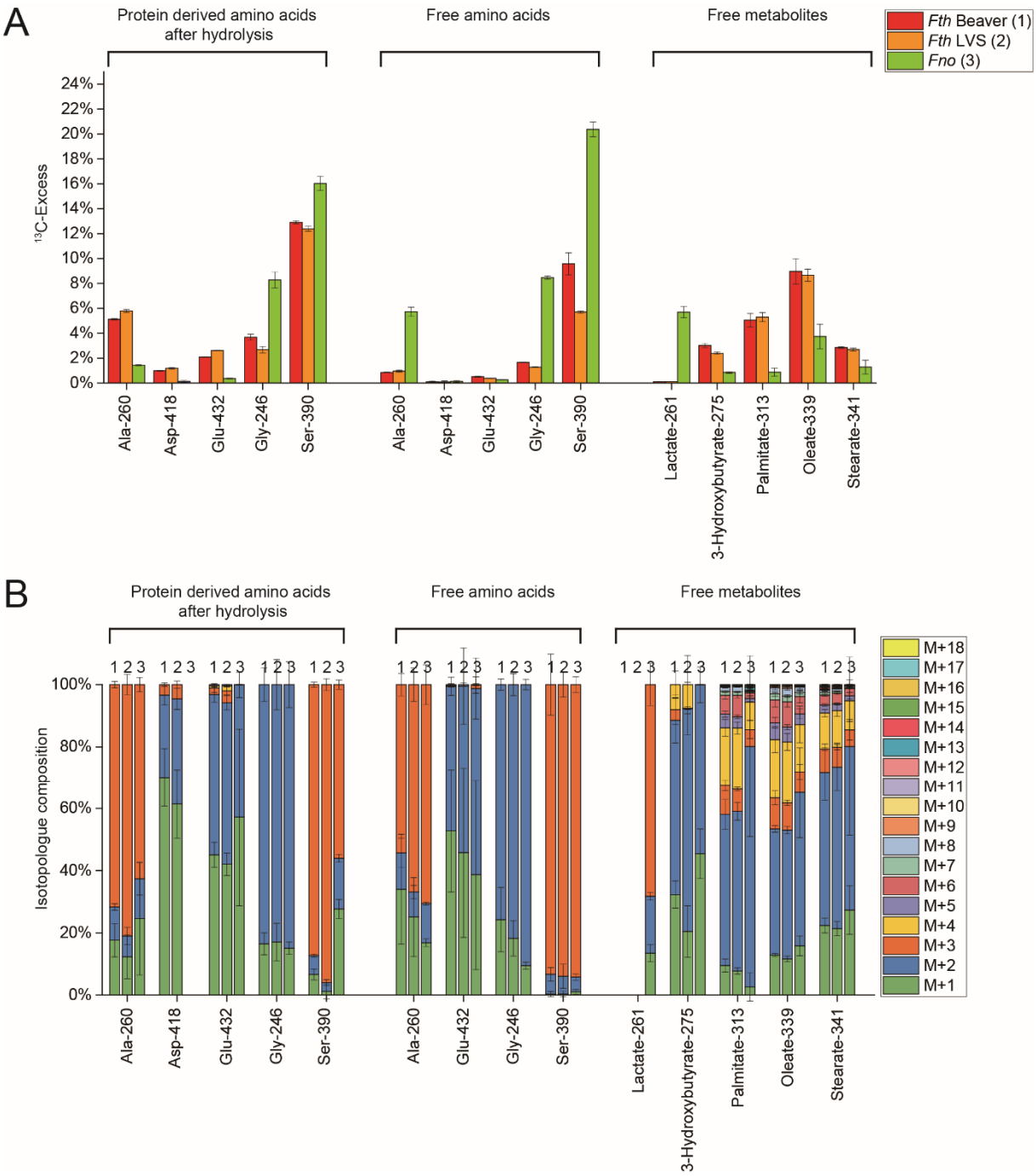


Figure 11. (A) ^{13}C-Excess (mol%) and (B) the fractional isotopolog distributions (%) in key metabolites of *Fth* Beaver (1), *Fth* LVS (2), or *Fno* (3) grown in medium T supplied with 3 mM [U-$^{13}C_3$]serine. For numerical values, see **Supplemental Tables S3**, **S7**, **S11**, **S15**, **S19**. For more details, see also legend to **Figure 6** (Chen et al., 2017).

3.1.4 Labelling experiments with [U-$^{13}C_3$]glycerol

From the ^{13}C-enrichment of methanol extracted glycerol (*Fth* strains, 93%; *Fno*, 59%) and the isotopolog distribution of the obtained glycerol (mainly M+3 labelled), it was concluded that [U-$^{13}C_3$]glycerol was efficiently incorporated into the bacterial cell (**Figure 12**). Via [U-$^{13}C_3$]pyruvate, Ala (protein derived and in the free form) was formed as indicated by the M+3 isotopologs. Via

[U-$^{13}C_2$]acetyl-CoA, the ^{13}C-flux can be measured into the TCA and fatty acids, as reflected by the M+2 isotopologs in Glu (derived from α-ketoglutarate), Asp (derived from oxaloacetate), succinate, malate, fatty acids and 3-hydroxybutyrate. Generally, the ^{13}C-excess values of different metabolites were much lower in *Fth* than those in *Fno* reflecting the better usage of glycerol in the less pathogenic *Francisella* strain *Fno*. Glycerol was also a better precursor for the products derived via glucogenesis and the PPP in *Fno*. Tyr (mainly as M+3 and M+2 from either PEP or the erythrose 4-phosphate precursor, respectively), Ser and Gly (via the glucogenic intermediate, 3-phosphoglycerate), cell wall derived amino sugars glucosamine and muramic acid in *Fno* were higher labelled than in *Fth*. The amino sugars were mainly M+3 labelled, but M+1 and M+2 was also present in substantial amounts (**Figure 12B**) reflecting the usage of glycerol for amino sugar biosynthesis. In summary, glycerol was efficiently used in *Fno*, but only at minor rates in *Fth* strains.

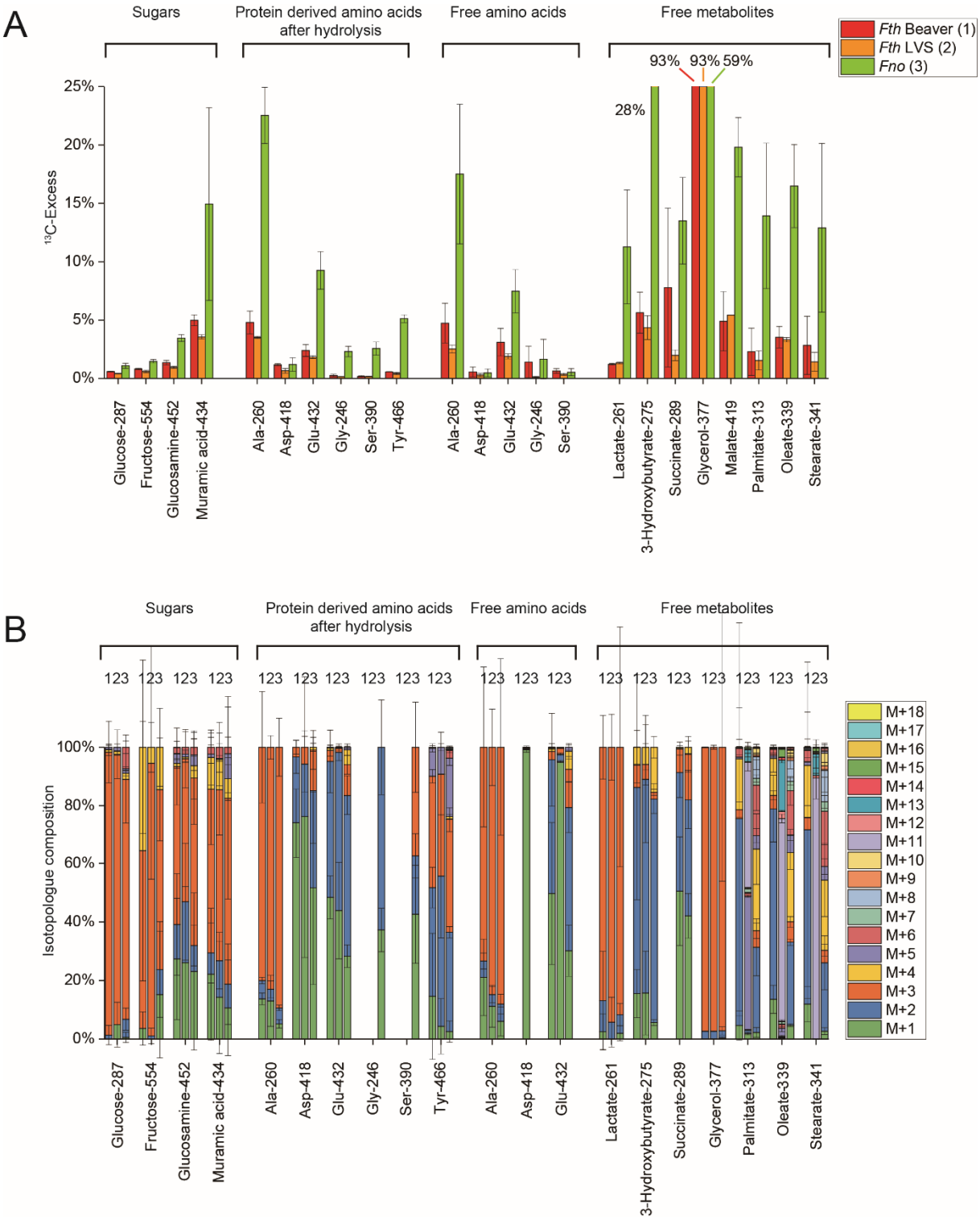


Figure 12. (A) ^{13}C-Excess (mol%) and (B) the fractional isotopolog distributions (%) in key metabolites of *Fth* Beaver (1), *Fth* LVS (2), or *Fno* (3) grown in medium T supplied with 25 mM [U-$^{13}C_3$]glycerol. For numerical values, see **Supplemental Tables S4**, **S8**, **S12**, **S16**, **S20**, **S24**. For more details, see also legend to **Figure 6** (Chen et al., 2017).

3.1.5 Differential substrate usage

In medium T (see also **Supplemental Tables S25**, **S26**) there were some poorly defined components

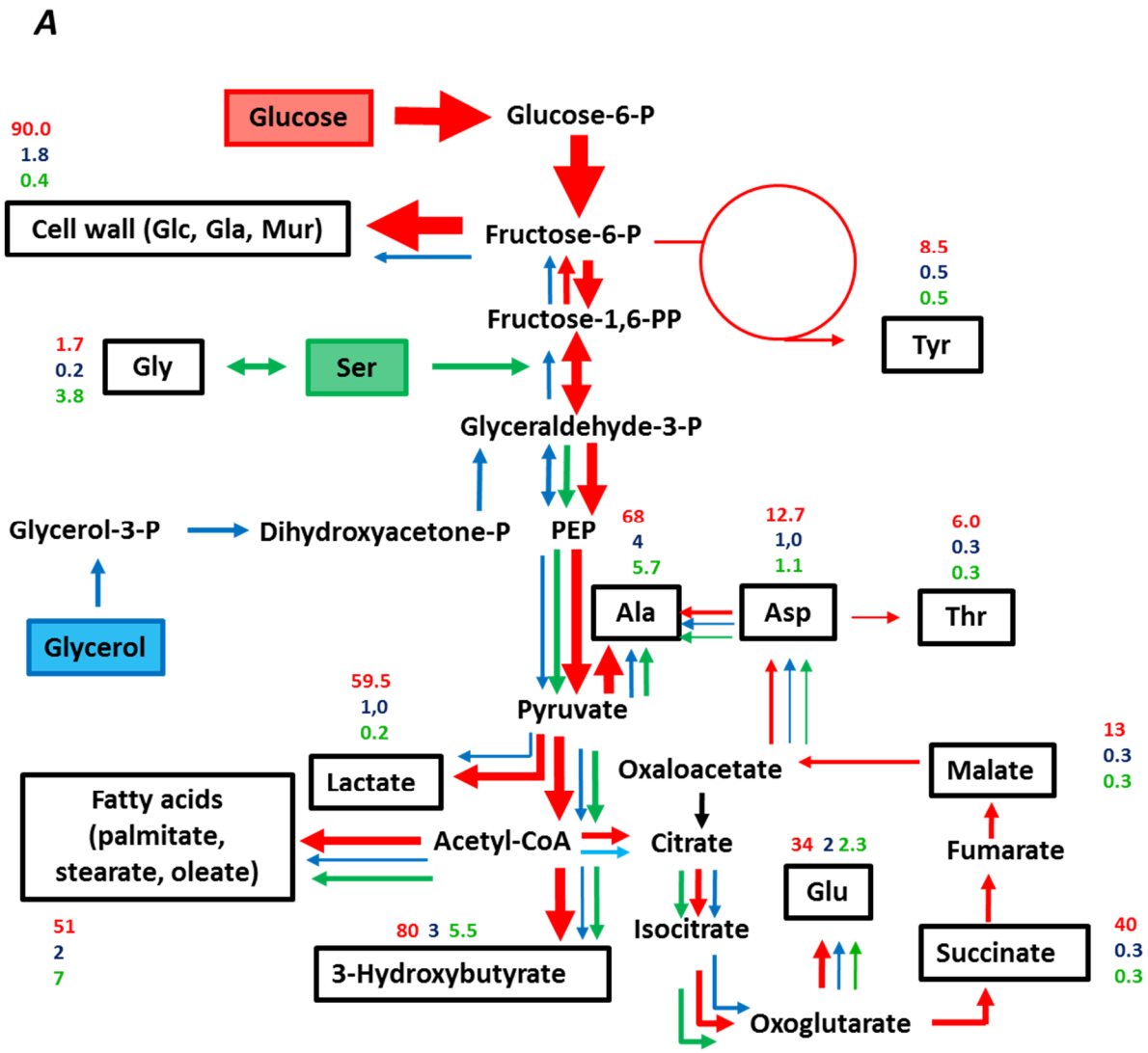
such as bacto tryptone (10 g/L), casamino acids (10 g/L), and brain heart infusion broth (10 g/L), so it was important to analyse the amounts of unlabelled glucose, serine and glycerol in this medium in an attempt to normalize the incorporation rates described above for the respective ^{13}C-tracers. Therefore, medium T (without the ^{13}C-tracers) was prepared and autoclaved. After lyophilisation, one fraction was silylated and analysed by GC-MS for quantifying the amounts of free glucose, glycerol, and amino acids, e.g., serine, respectively. Another fraction was dissolved and hydrolysed in 6 M hydrochloric acid at 105 °C, the hydrolysate was lyophilized, derivatized and again analysed by GC-MS in order to also quantify the protein- or peptide- bound serine.

In the medium, free glucose could be detected, in amounts of 75.3 mM. However, free glycerol could not be detected and free serine was present at a concentration of 0.5 mM. The total serine was present at a concentration of 3.1 mM after the hydrolysis of peptides. On this basis, in the labelling experiments the total concentration of glycerol was 25 mM (with 100% [U-$^{13}C_3$]glycerol), glucose was present at a total concentration of 94.4 mM (with 11.8% [U-$^{13}C_6$]glucose), and the overall concentration of serine (free form and in peptides) was 3.4 mM (with 84.3% [U-$^{13}C_3$]serine) (**Supplemental Tables S25**, **S26**).

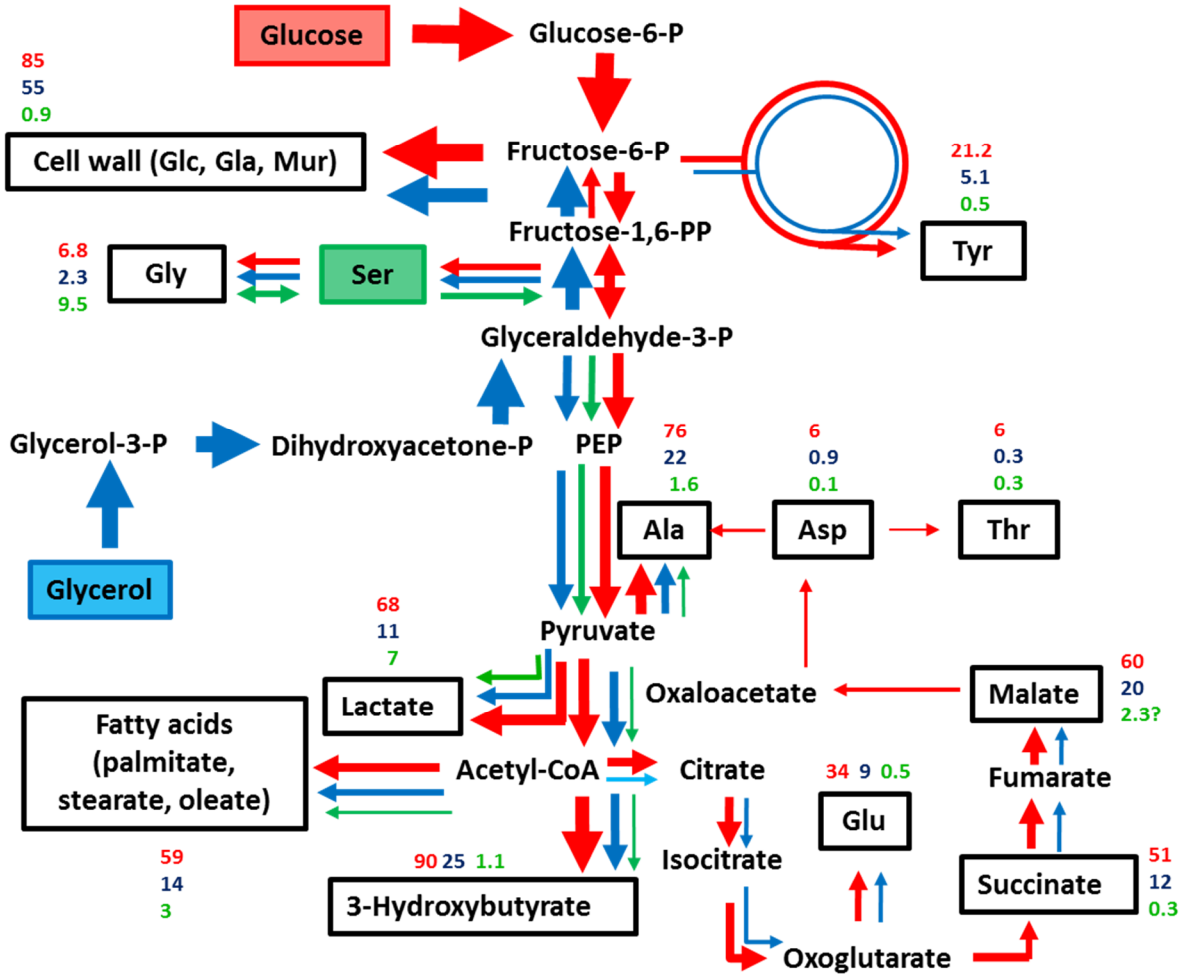
On the basis of these findings, we normalized the ^{13}C-enrichments from the different tracers. For [U-$^{13}C_6$]glucose labelling experiments, the values were multiplied by a factor of 8.5, for the [U-$^{13}C_3$]serine labelling experiments by a factor of 1.15 and for the [U-$^{13}C_3$]glycerol labelling experiments by a factor of 1. The normalized values are shown in **Figure 13A** and **13B** for *Fth* and *Fno*, the relative fluxes are also indicated in **Figure 13** by the arrow widths. It was obvious that the glycogen and cell wall amino sugars were mainly formed from glucose in the medium T (>85%). The ^{13}C-label was incorporated predominately from glucose into alanine and fatty acids (>50%). In *Fth* and *Fno*, the ^{13}C-fluxes from serine into the metabolism were lower. Using glycerol as precursor, the ^{13}C-fluxes were lower in the *Fth* strains. In *Fno*, however, the ^{13}C-fluxes were higher into the biosynthesis (up to 55% in cell wall sugars).

The differences between the metabolic fluxes in *Fth* strains and *Fno* are shown in **Figure 13C**. The numbers indicate the differences between the normalized ^{13}C-enrichments in *Fth* and *Fno* (*Fth–Fno*). Higher fluxes in *Fth* are indicated by normal arrows, whereas lower fluxes are shown by dashed arrows. In *Fth*, the flux from glucose into the cell wall was slightly higher, but showed reduced fluxes (with the exception of Asp for unknown reasons) into any other metabolite under study. Moreover, the fluxes from serine into downstream pathways in *Fth* strains were higher (i.e., the lower part of EMP, formation of acetyl-CoA and fatty acids). However, there was no flux from serine into cell wall sugars,

which suggests that there was a more “bipartite metabolic flux” in *Fth* strains (i.e., with glucose feeding directly the formation of polysaccharides, and serine adding more carbon for pyruvate and actyl-CoA metabolism) comparing with the less less human-pathogenic *Fno*. The glycerol uptake in *Fth* strains was not so efficient as that in *Fno*, which indicated that the glucogenesis from glycerol was not a major process in *Fth* strains.



B


Glucose
Glucose-6-P
85
55
0.9
Cell wall (Glc, Gla, Mur)
Fructose-6-P
21.2
5.1
0.5
Tyr
Fructose-1,6-PP
6.8
2.3
9.5
Gly
Ser
Glyceraldehyde-3-P
Glycerol-3-P
Dihydroxyacetone-P
PEP
76
22
1.6
6
0.9
0.1
6
0.3
0.3
Ala
Asp
Thr
Glycerol
68
11
7
Pyruvate
Lactate
Oxaloacetate
Malate
60
20
2.3?
Fatty acids
(palmitate,
stearate, oleate)
Acetyl-CoA
Citrate
34 9 0.5
Glu
Fumarate
59
14
3
90 25 1.1
3-Hydroxybutyrate
Isocitrate
Succinate
51
12
0.3
Oxoglutarate

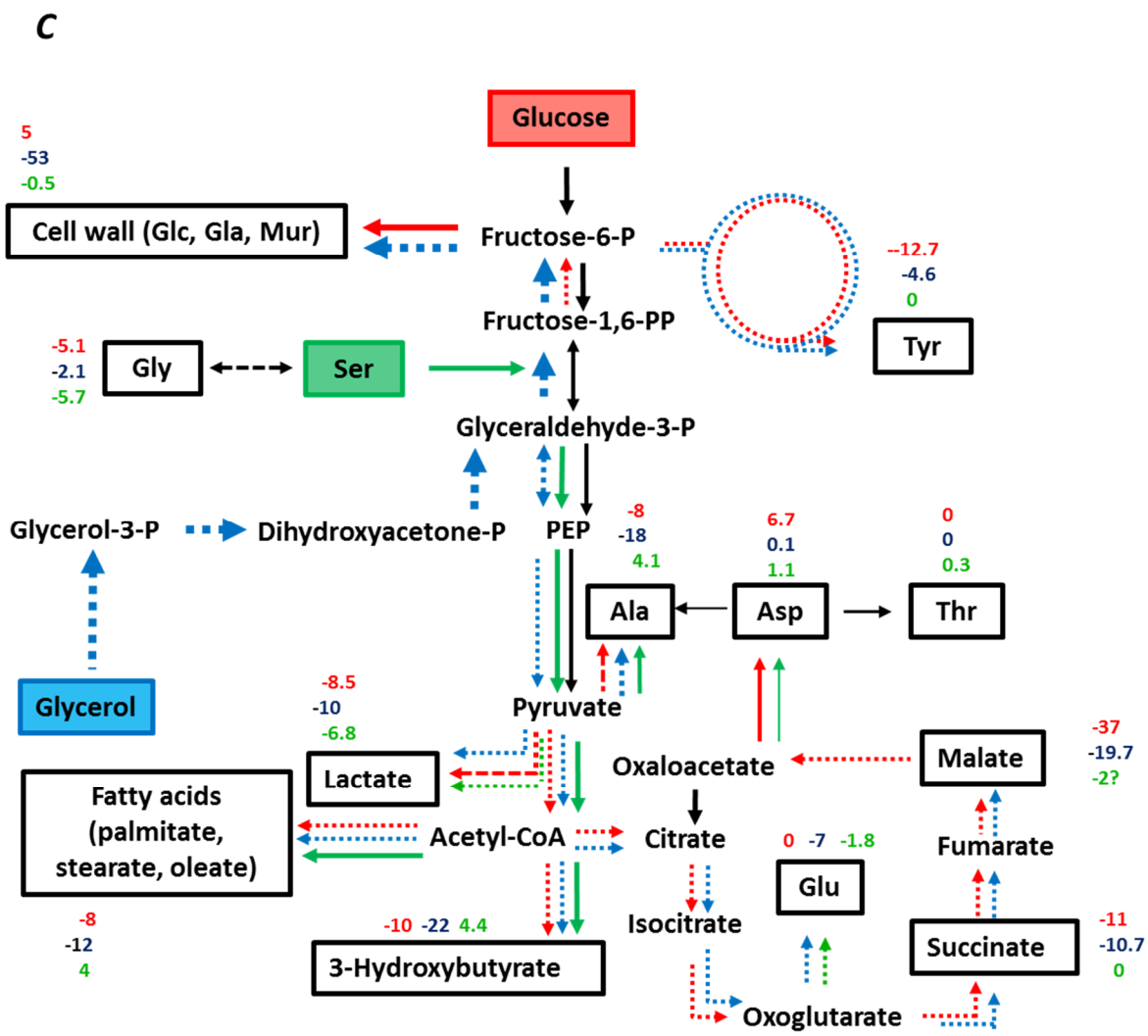


Figure 13. Metabolic pathways and fluxes in (A) *Fth* Beaver/*Fth* LVS, and (B) *Fno* from exogenous glucose (red arrows), serine (green arrows), and glycerol (blue arrows). (C) Differences in the metabolic fluxes between *Fth* and *Fno* (*Fth–Fno*). Metabolites studied by GC-MS-based isotopologue profiling are indicated by black boxes. The numbers indicate normalized overall ^{13}C-enrichments (from labeled glucose, glycerol and serine in red, blue and green, respectively). The arrow widths roughly indicate the relative fluxes. Fluxes conducive to enrichments <1% are not shown. In (C) the numbers indicate the differences in the normalized overall 13C-enrichments (*Fth–Fno*). Higher fluxes in *Fth* are indicated by normal arrows, lower fluxes by dashed arrows (Chen et al., 2017).

3.1.6 Discussion

The pathogenic *Francisella* strains can be grown in the cytosolic compartments of various host cells (Santic et al., 2006; Sjostedt, 2006; Keim et al., 2007). Therefore, *Francisella* strains could benefit from versatile and changing nutrient supplies from the host cells, establishing a high degree of metabolic robustness and adaptation capacities of interacellular *Francisella* strains. On the basis of the genomes of *Francisella* strains, it can be assumed that amino acids, glucose and glucogenetic substrates (e.g., glycerol or pyruvate) could be the major carbon substrates feeding a highly

interconnected metabolic network. Earlier work also confirmed the versatile role of amino acids, glucose, and glycerol as nutrients (Checroun et al., 2006; Meibom and Charbit, 2010; Santic and Abu Kwaik, 2013; Barel et al., 2015).

3.1.6.1 Glucose

The phosphofructokinase was not annotated within the genomes of *Ftt*, *Fth*, and *Fno* until 2012. Therefore, it was suggested that gluconeogenesis and not glycolysis is important for intracellular replication (Meibom and Charbit, 2010; Raghunathan et al., 2010). The in vitro and in vivo growth was shown to be hampered with the knocking out of GlpX (Kadzhaev et al., 2009; Brissac et al., 2015). And from earlier work of Kingry and Petersen, the carbohydrate metabolism suggests to be different between the human pathogenic *Ftt* and *Fth* species and the mice pathogenic *Fno* species (Kingry and Petersen, 2014). *Fth* could metabolise glucose, however, could not use glucose 6-phosphate as a precursor in vitro. In the work of Gyuranecz, only *F. hispaniensis* and *F. philomiragia* could metabolise glucose 1-phosphate and glucose 6-phosphate (Gyuranecz et al., 2010). In the *Fno* strain, phosphofructokinase (FTN_1210) was found, which indicated the function of glycolysis in *Fno* (Enstrom et al., 2012, Brissac et al., 2015).

From our research data, it is obvious that glucose is the most efficient substrate for the *Francisella* strains in medium T. Differences between the metabolism of *Fth* WT and LVS were not significant, which suggests that the *Fth* LVS is not (strongly) related to glucose usage. From **Figure 13C,** it was concluded that the cell wall sugar formation in *Fth* strains was slightly more efficient than that in *Fno*. Either *Fth* strains or *Fno* degraded glucose via glycolysis but not the ED or the PPP. The carbon flux from glucose to amino acids and the TCA cycle in *Fth* strains was lower than in *Fno*, which suggested that *Fth* metabolised glucose for generating energy by a bipartite metabolic network as in *L. pneumophila* (Gillmaier et al., 2016; Häuslein et al., 2016) and *Chlamydia trachomatis* (Mehlitz et al., 2017). With the labelling data of alanine, we could also demonstrate that an Ala dehydrogenase or Glu/Pyr transaminase must be present, although the genes for both enzymes are not annoted in the genome. Moreover, our work also showed the expected activity of an aspartate 4-decarboxylase.

3.1.6.2 Glycerol

In *Fno*, glycerol is mainly metabolised via the EMP pathway during the intracellular replication (Kadzhaev et al., 2009; Brissac et al., 2015), and the enzyme GlpD (glycerol 3-phosphate dehydrogenase) is very important for glycerol degradation (Brissac et al., 2015).

Our results verified the earlier work (Petersen and Schriefer, 2005; Marinov et al., 2009; Gyuranecz et al., 2010; Huber et al., 2010), but also showed more detail about the glycerol metabolism. The carbon flux was much higher in the mouse pathogenic strain *Fno* than that in the highly pathogenic *Fth* strains. For *Fno*, the uptake of glycerol was very efficient and the glycerol was well used as substrate for gluconeogenesis even in the presence of glucose.

In *Fth* strains, the ^{13}C-enrichment of muramic acid was 4-5% and alanine was also labelled using [U-$^{13}C_3$]glycerol as precursor. These finding indicated that *Fth* strains could also metabolise glycerol. In earlier work, the fermentation and metabolism of glycerol in *Fth* strains were negative, but the *Fth* strains could metabolise glycerol phosphate (Petersen and Schriefer, 2005; Gyuranecz et al., 2010; Huber et al., 2010). The glycerol phosphate transporter GlpT could be the reason for this. However, in the *Francisella* genomes, putatively encoding a *glpF* gene was also annotated (Raghunathan et al., 2010). Moreover, in 2009 Marinov and his co-workers published a report in which the acid formation from glycerol in three *Francisella* strains was described. Because of these earlier studies, the metabolism of glycerol is no more a surprise. But the low utilization of glycerol in *Fth* strains with the presence of all genes for glycerol metabolism is still unclear. In both *Fth* strains, however, *gfpK* and *glpD* are separated by an IS-Ftu1 element, the operon is disconnected.

3.1.6.3 Serine

Amino acids (e.g. Ser) are important for the intracellular replication of *Francisella* strains (Meibom and Charbit, 2010; Raghunathan et al., 2010; Barel et al., 2012, 2015; Steele et al., 2013; Brown et al., 2014; Ramond et al., 2014; Gesbert et al., 2015). A report of Barel and his co-workers also showed that the the SLC family of host amino acid transporters is important for intracellular replication of Fth strain LVS (Barel et al., 2012). Interestingly, in the close relative *L. pneumophila*, a human pathogenic bacterium which replicates within a vacuole in alveolar macrophages, a similar finding of the usage of amino acids during intracellular growth and the involvement of SLC proteins of the host cell was found (Wieland et al., 2005; Eisenreich and Heuner, 2016).

From our in vitro study of *Francisella* strains, the carbon flux from Ser into the downstream metabolites in *Fth* and especially in *Fno* was quite low, even lower was the carbon flux via gluconeogenesis in both species (as gleaned e.g., from the apparently unlabelled cell wall-derived carbohydrates). On the other hand, the glycerol experimental data showed that the carbon flux could be directed into the gluconeogenesis. Surprisingly, however, this flux could not be seen with serine as

a glycogenic substrate (**Figures 13A**, **B**).

The degradation of Ser to form some protein derived amino acids was more efficient in *Fth* strains than in *Fno*. However, the formation of Gly from Ser by glycine hydroxymethyltransferase was lower in *Fth* strains, which suggested that the glycine cleavage system (GCS) is important for replication in Ser-limiting environments and that a *gcvT* mutant strain is auxotrophic for Ser. This indicates the importance of the Ser to Gly (and vice versa) converting systems (Meibom and Charbit, 2010; Brown et al., 2014). Earlier work of Ravnikar and Somerville showed that the disrupted Ser biosynthesis pathway indicated the glycine-dependent Ser production in *E.coli* (Ravnikar and Somerville, 1987). In *Fth* strains, the only source of Ser biosynthesis is GCS, which was also found surprisingly in the *Ftt* strain (Brown et al., 2014). Because of a pseudo *serB* gene (P-serine phosphatase), the biosynthesis pathway from 3-PG to Ser in *Fth* strains is incomplete, which also confirmed our results that there was no carbon flux from glucose or glycerol to Ser.

The isotopologue profiling of protein derived Ser indicated the formation of Gly. However, this was not true for free Ser, which represented the stationary phase. The free amino acids (Ala, Gly and Ser) and lactate were higher labelled in *Fno* than in *Fth* strains. In opposite the protein derived amino acids in the *Fth* strain were more labelled than in *Fno*. This might reflect that Ser was more efficiently used in the replication phase and less used in the stationary phase during the *Fth* strains' growth. Similar results were also found in *L. pneumophila* (Gillmaier et al., 2016).

Together, the data provide evidence for a bipartite metabolism in *Fth* strains, similar, but not identical to *L. pneumophila*, where amino acids were used as carbon and energy source in the exponential phase and glucose was additionally used in the post-exponential phase (Eisenreich and Heuner, 2016; Gillmaier et al., 2016; Häuslein et al., 2016).

In conclusion, our findings confirm the multiple usage (glucose, glycerol and serine) by *Francisella* strains. We speculate that the metabolic differences of *Fth* strains and *Fno* could be related to their host-specific virulence. This hypothesis should now be analysed by *in vivo* studies of *Francisella* strains in various host cells.

3.2 Differential substrate usage and metabolic fluxes in *Francisella* W12-1067 WT and Δmyo-Inositol mutant

3.2.1 Experimental approach

Similar experiments as described in **3.1** were done with *Francisella* W12-1067 WT (Rydzewski et al., 2014) and its Δmyo-Inositol mutant using the ^{13}C-labelled substrates, [U-$^{13}C_6$]glucose, [U-$^{13}C_3$]serine and [U-$^{13}C_3$]glycerol, and the ^{2}H-labelled substrate myo-inositol-C-d_6. For the ^{13}C-labelling experiments, each strain was grown in medium T supplemented with 11 mM [U-$^{13}C_6$]glucose, 11 mM [1,2-$^{13}C_2$]glucose, 3 mM [U-$^{13}C_3$]serine or 25 mM [U-$^{13}C_3$]glycerol at 37 ^{o}C for 26 h. After that, the cells were harvested at an OD600 value of approximately 1.8, then the cells were washed with PBS solution twice, centrifuged, pelleted, autoclaved and lyophilized to get about 100 mg of dried cell pellet. For ^{2}H-labelling experiments, each strain was first grown in medium T overnight, then grown in CDM medium supplemented with 9.13 mM myo-inositol-C-d_6, and harvested at a maximal OD600 value (after about 50 h). Then, the cells were washed with PBS solution twice, centrifuged, pelleted, autoclaved and lyophilized to get about 100 mg of dried cell pellet. Using physical or chemical procedures, different sugars from the cell wall or glycogen, protein derived or free amino acids, and free polar metabolites were isolated and converted into various nonpolar derivatives which were later analysed by GC/MS spectrometry (for details, see **Materials and Methods**). The labelling experiments were repeated at least once (biological replicate), and each sample was analysed three times by GC/MS spectrometry (technical replicates). Based on the biological replicates and technical replicates, six experimental values of each metabolite were obtained. Mean values and standard deviations of each metabolite were then calculated (see also **Supplemental Tables S28-51** and **S90-95** for numerical data).

3.2.2 Analysis of main pathways for the metabolism of glucose, glycerol and serine of *Francisella* sp. W12-1067 grown in medium T

3.2.2.1 Labelling experiments with [U-$^{13}C_6$]glucose

3.2.2.1.1 Isotopolog profiles of sugars

Glucose from polysaccharides and glucosamine and muramic acid from cell walls were isolated as described above. Free fructose could be extracted from the dried cell pellets using water. All sugars showed ^{13}C-incorporation at about 10% ^{13}C (**Figure 14A**, **Supplement Table S36**). As described for *Fno*, these high ^{13}C-enrichments reflect the efficient uptake of glucose also in W12-1067. The main isotopolog in glycogen-derived glucose and free fructose was the M+6 species (reflecting six

^{13}C-atoms in the same molecule) which can be explained by the direct incorporation of the supplied [U-$^{13}C_6$]glucose into glycogen or fructose (**Figure 14B**, **Supplement Table S48**). The higher fractions of M+1 to M+5 in cell wall derived amino sugars (70-90%) than that of glycogen derived glucose (20%) could again reflect metabolic turnover of glucose phosphate prior to its usage for cell wall synthesis.

3.2.2.1.2 Isotopolog profiles of protein derived amino acids

Protein derived amino acids were also obtained from acidic hydrolysates of the bacterial pellets at high yields. For GC/MS analysis, these amino acids were converted into their TBDMS-derivatives. The measured ^{13}C enrichments ranked from Ala > Glu > Ser > Tyr (**Figure 14A**). The other amino acids were apparently unlabelled probably due to the presence of unlabelled amino acids and peptides in the complex medium T containing brain heart infusion broth, bacto trypton and casamino acids (see also **Supplement Table S26**).

On the basis of the labelling profiles, some conclusion can be made. [U-$^{13}C_6$]glucose was efficiently degraded to [U-$^{13}C_3$]pyruvate, which is reflected by the observation of [U-$^{13}C_3$]Ala. However, Ala carried also one or two ^{13}C-atoms, as reflected by the M+1 and M+2 mass peaks, respectively. These isotopologues can be explained by the decarboxylation of M+1 and M+2 Asp resulting in [$^{13}C_1$]- or [$^{13}C_2$]pyruvate/Ala, respectively. The relative contributions can be determined by the quantitative analysis of the mass spectra and it turned out that, similarly as in *Fth*, Ala was mainly (65%) formed from [U-$^{13}C_3$]pyruvate and 35 % were derived via the Asp route. The high fractions of M+1 and M+2 and lower fractions of M+3, M+4 and M+5 in Glu indicated high turnover rates between oxaloacetate and α-ketoglutarate in the TCA cycle. The high fractions of [U-$^{13}C_3$]Ser (M+3) in the fragment containing all three carbon atoms of the original serine molecule (Ser-390) indicated efficient usage of glucose present in medium T and the formation of [U-$^{13}C_3$]3-phosphoglycerate by degradation of [U-$^{13}C_6$]glucose. The labelling profiles of Tyr were more complicated and contained M+1 to M+9 fractions. That could be explained by the formation of erythrose 4-phosphate via the PPP and PEP via glycolysis, as degradation products of [U-$^{13}C_6$]-glucose.

3.2.2.1.3 Isotopolog profiles of polar metabolites

Using methanol as a solvent, more than 20 polar metabolites including free amino acids, TCA intermediates, and free fatty acids were extracted from the dried bacteria pellet. The ^{13}C-excess values and isotope distributions of free amino acids were similar to those of protein-derived amino acids. Free fatty acids and 3-hydroxybutyrate were highly labelled (6-8%, **Supplement Table S32**), suggesting

that the degradation product acetyl-CoA acquired the ^{13}C-label of glucose at high rates. Succinate was formed from acetyl-CoA via the TCA, therefore it was mainly M+1 and M+2 labelled. Lower fractions of M+3 labelled succinate could indicate fluxes via oxaloacetate made by carboxylation of M+3 pyruvate.

In total, glucose was an efficient substrate for the formation of fructose, glycogen, cell wall, some amino acids, and fatty acids by carbon flux via glycolysis (see below), acetyl-CoA formation and its usage for filling the TCA cycle and further downstream products (e.g. fatty acids). In comparison to our earlier findings for other *Francisella* strains, W12-1067 resembled *Fno* in its glucose usage.

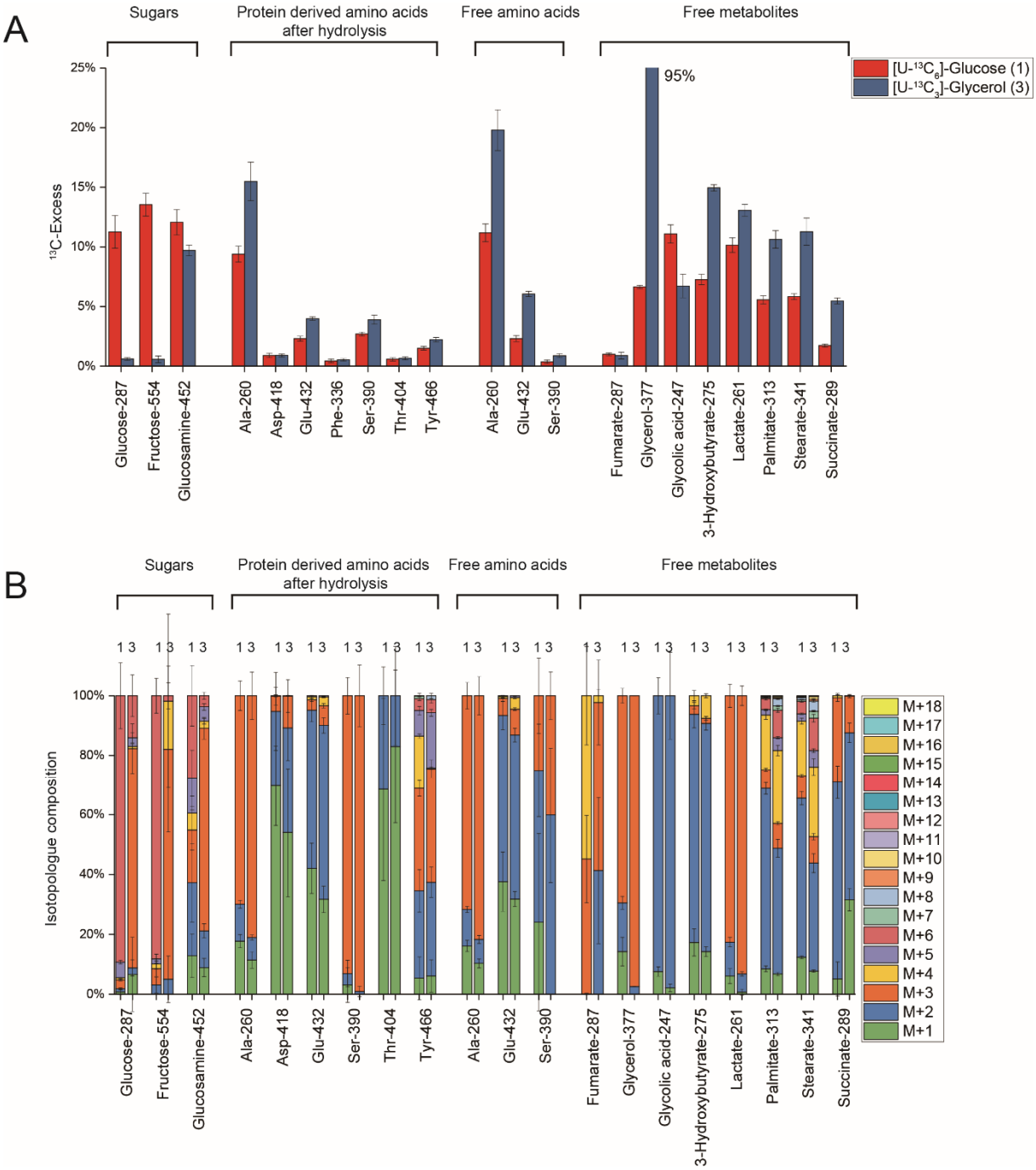


Figure 14. (A) ^{13}C-Excess (mol%) and (B) the fractional isotopolog distributions (%) in key metabolites of *Francisella sp.* W12-1067 grown in medium T supplied with 11 mM [U-$^{13}C_6$]glucose or 3 mM [U-$^{13}C_3$]serine or 25 mM [U-$^{13}C_3$]glycerol. 13C-Excess (mol%) and relative fractions of isotopologs (%) were determined by GC/MS of the derivatives of different metabolites at the indexed m/z values. Error bars indicate standard deviations from the means of 6 to 9 values 2–3 × biological replicates, 3 × technical replicates. M+1, M+2, M+3, etc. indicate isotopologs carrying 1, 2, 3, etc. ^{13}C-atoms. For numerical values, see **Supplemental Tables S28-51**.

3.2.2.2 Labelling experiments with [1,2-$^{13}C_2$]glucose

In order to specify that glucose was indeed degraded via glycolysis, we performed additional

experiments with [1,2-$^{13}C_2$]glucose. The conversion of [1,2-$^{13}C_2$]glucose via the Entner-Doudoroff pathway or glycolysis is conducive of specific labelling patterns in pyruvate/Ala. Specifically, [1,2-$^{13}C_2$]pyruvate/alanine would result from [1,2-$^{13}C_2$]glucose following the Entner-Doudoroff pathway, whereas [2,3-$^{13}C_2$]pyruvate/alanine would reflect glucose degradation via glycolysis. To distinguish between the two scenarios, we have used a thorough analysis of the Ala-232 fragment (containing C-2 and C-3 of the original alanine molecule). Indeed, Ala-232 was found M+2 labelled at the same rate as in Ala-260 indicating that C-2 and C-3 of Ala carried the ^{13}C-label (**Figure 15**, see also **Supplement Table S41**). Thus, glucose was degraded via glycolysis (EMP pathway) and not via the Entner-Doudoroff pathway in *Francisella* sp. W12-1067.

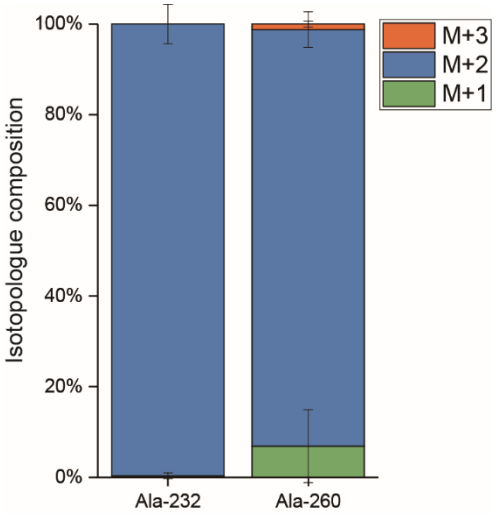


Figure 15. Transfer of ^{13}C-label from [1,2-$^{13}C_2$]glucose into alanine of *Francisella* sp. W12-1067. Fractional isotopolog distributions (%) in the silylated fragments Ala-260 and Ala-232 carrying C_1-C_3 and C_2-C_3 of the original alanine carbon chain, respectively.

3.2.2.3 Labelling experiments with [U-$^{13}C_3$]serine

[U-$^{13}C_3$]serine could be taken up by W12-1067, which was clearly seen by the ^{13}C-enrichments in methanol extracted free serine (4%) and protein derived serine (14%) (see **Supplemental Table S30, S34**). The observed labelling profiles (**Figure 14**) suggested that serine was degraded to form pyruvate, which subsequently was converted into alanine or acetyl-CoA for e.g. fatty acid biosynthesis. However, serine was not effectively used for gluconeogenesis, as shown by the low incorporation into Phe and Tyr, and into the sugars (glucose from glycogen, free fructose and amino sugars from cell wall).

3.2.2.4 Labelling experiments with [U-$^{13}C_3$]glycerol

For methanol extracted polar metabolites, it was found that glycerol was highly labelled (93%, see **Supplemental Table S35**), which indicated the efficient uptake of glycerol during the cultivation of W12-1067. [U-$^{13}C_3$]glycerol was metabolized into [U-$^{13}C_3$]pyruvate which formed the detected [U-$^{13}C_3$]alanine and [U-$^{13}C_3$]lactate. Via [U-$^{13}C_2$]acetyl-CoA, Asp, Glu, TCA intermediates and free fatty acids mainly acquired a M+2 signature. Due to the conversion of Asp into pyruvate, Ala and lactate were also found as M+1 and M+2 isotopologues (**Figure 14B**). Tyr was predominantly M+2 and M+3 labelled, which indicated the utilization of [U-$^{13}C_3$]glycerol via glucogenesis and PPP to form PEP and erythrose 4-phosphate, respectively. Notably, cell wall amino sugars (glucosamine and muramic acid) were highly labelled, which again provides evidence that glycerol serves a good substrate to form hexose phosphate required for cell wall biosynthesis. However, free fructose and glycogen derived glucose were almost unlabelled, suggesting that unlabelled glucose from the medium could be more efficiently shuffled to these sugars.

3.2.2.4 Differential substrate usage

As in our work to analyse the main pathways for the metabolism of glucose, glycerol and serine of *Francisella* strains grown in medium T (Chen et al., 2017), we now normalized the ^{13}C-enrichments according to the presence of unlabelled glucose, glycerol and serine in free or polymeric form in the components of medium T. On this basis, the values from the experiment with [U-$^{13}C_6$]glucose were multiplied by a factor of 8.5, from the experiment with [U-$^{13}C_3$]serine by a factor of 1.15, and from the experiment with [U-$^{13}C_3$]glycerol by a factor of 1 (see above). The normalized values are shown in **Figure 16**. It is obvious that exogenous glucose is most efficiently used for glycogen and cell wall amino sugars (>= 10%). However, significant carbon flux could also be detected from glucose via glycolysis and downstream processes into alanine, 3-hydroxybutyrate and free fatty acids. The carbon fluxes from serine and glycerol into the central metabolism were lower (for serine <5%, for glycerol <15%).

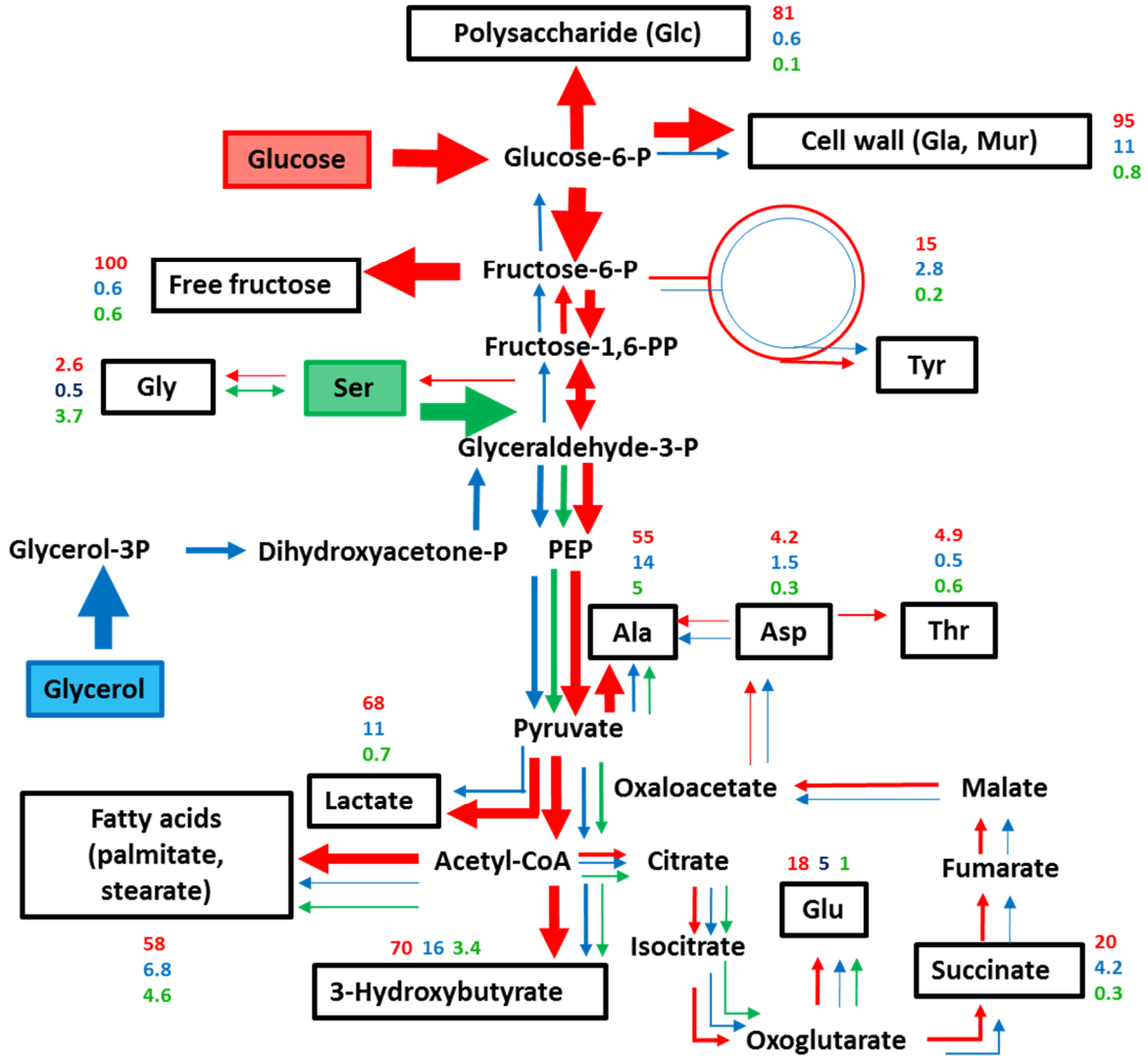


Figure 16. Observed metabolic pathway and flux in *F.* W12-1067 from glucose (red), serine (green) and glycerol (blue). The numbers present the normalized ^{13}C-excesses and the arrow widths indicate the relative fluxes.

3.2.3 Analysis of main pathways for the metabolism of glucose and glycerol of *Francisella* sp. W12-1067 Δmyo-Inositol mutant grown in medium T

To analyse the main pathways for the metabolism in the Δmyo-inositol mutant of *Francisella* sp. W12-1067, we performed additional experiments with this mutant growing in medium T supplied with 11 mM [U-$^{13}C_6$]glucose or 25 mM [U-$^{13}C_3$]glycerol. Using the same procedures as described above for the W12-1067 WT strain, we again analysed the isolated sugars from glycogen and cell walls, amino acids derived from proteins and free metabolites. Based on the averages from the values for biological replicates and technical replicates, the ^{13}C-excess values (mol%) and the fractional isotopologue distributions (%) are shown in **Figure 17**.

3.2.3.1 Labelling experiments with [U-$^{13}C_6$]glucose

As in the WT, glucose from glycogen, cell wall derived amino sugars and free fructose were highly labelled (about 10-12%, **Figure 17A**, **Supplement Table S36**). The major isotopologue of glycogen-derived glucose and free fructose was M+6 by conversion the [U-$^{13}C_6$]glucose into [U-$^{13}C_n$]glycogen and [U-$^{13}C_6$]fructose. Cell wall derived amino sugar glucosamine had again higher fractions of M+1 to M+5 (about 70%) than glycogen derived glucose and free fructose (about 10%, **Figure 17B**, **Supplement Table S48**). The ranking of ^{13}C enrichments in protein-derived amino acids (Ala > Ser > Glu > Tyr) slightly differed from the order in the WT. Ala was mainly M+3 labelled, which could be explained by glucosene degradation to [U-$^{13}C_3$]pyruvate. The high fractions of M+1 and M+2 in Glu could be reflect that Glu was predominantly formed from M+1 and M+2 α-ketoglutarate via M+1 and M+2 acetyl-CoA. Ser was formed from [U-$^{13}C_6$]glucose via [U-$^{13}C_3$]3-phosphoglycerate. Therefore, it was mainly M+3 labelled. Because of the high metabolic turnover in the PPP, the labelling profile of Tyr was more complicated with main fractions of M+2 and M+3 species. The ^{13}C-excess (mol%) and the fractional isotopolog distributions in free amino acids were almost the same as in protein derived amino acids. Because of the highly labelled acetyl-CoA, free fatty acids and 3-hydroxybutyrate acquired the label at rates of 5-7% (**Supplement Table S32**). Lactate was also highly labelled (10%), reflecting its formation from [U-$^{13}C_6$]glucose via [U-$^{13}C_3$]pyruvate.

3.2.3.2 Labelling experiments with [U-$^{13}C_3$]glycerol

Using [U-$^{13}C_3$]glycerol as a precursor, free fructose and glycogen derived glucose were almost not labelled. However, glucosamine was highly labelled (10%, **Figure 17A**, **Supplement Table S39**), which could again be explained by gluconeogenetic carbon flux from glycerol into glucose 6-phosphate. [U-$^{13}C_3$]glycerol was also metabolized into [U-$^{13}C_3$]pyruvate, which later formed [U-$^{13}C_3$]alanine and [U-$^{13}C_3$]lactate. [U-$^{13}C_3$]pyruvate can be again degraded to form [U-$^{13}C_2$]acetyl-CoA. Consequently, Glu, TCA intermediates and free fatty acids were mainly M+2 labelled via [U-$^{13}C_2$]acetyl-CoA.

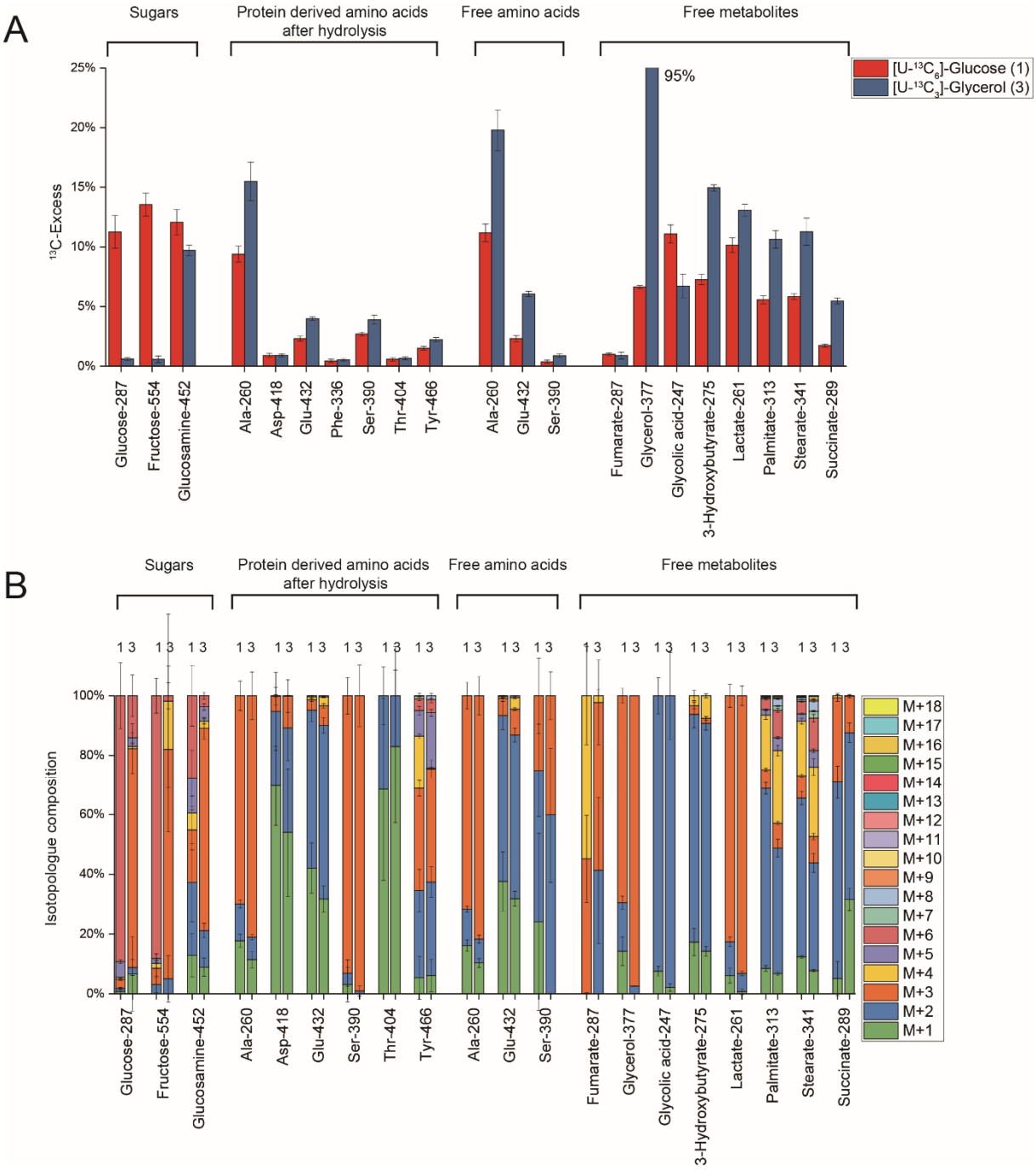
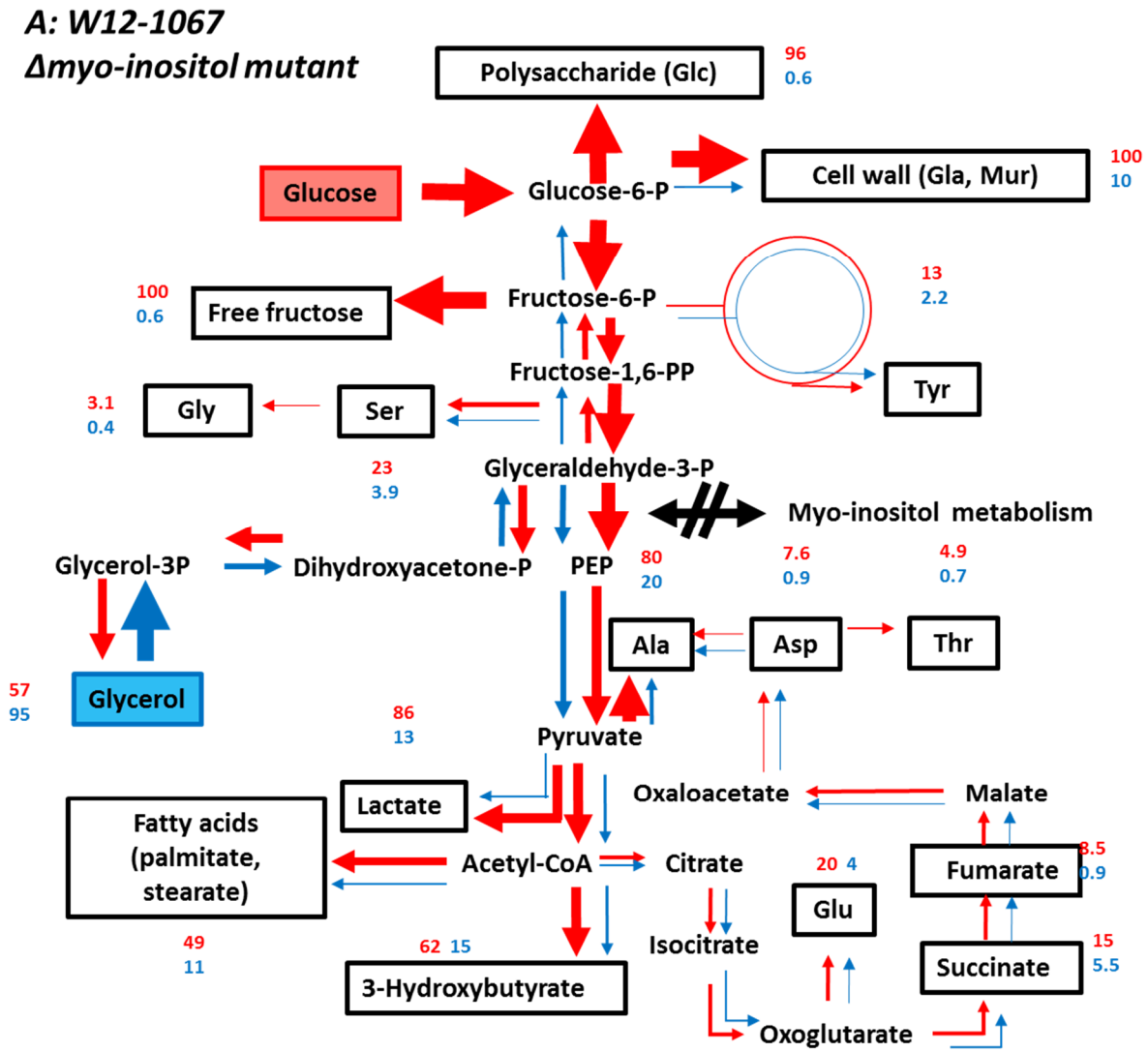


Figure 17. (A) ^{13}C-Excess (mol%) and (B) the fractional isotopolog distributions (%) in key metabolites of *Francisella sp.* W12-1067 Δmyo-Inositol mutant grown in medium T supplied with 11 mM [U-$^{13}C_6$]glucose or 25 mM [U-$^{13}C_3$]glycerol. ^{13}C-Excess (mol%) and relative fractions of isotopologs (%) were determined by GC/MS of the derivatives of different metabolites at the indexed m/z values. Error bars indicate standard deviations from the means of 6 values 2 × biological replicates, 3 × technical replicates. For numerical values, see **Supplemental Tables S28-51**.

3.2.4 Differences between the ^{13}C-excess of *F.* W12-1067 Δmyo-inositol mutant and WT

Figure 18(A) presents the normalized carbon fluxes for the Δmyo-inositol mutant of *Francisella* sp. W12-1067. The fluxes indicate that glucose is mainly incorporated into glycogen, cell wall amino

sugars, alanine and fatty acids. Lower fluxes are shown from glycerol into alanine, lactate and fatty acids. To visualize the differences between the metabolic fluxes of the myo-inositol mutant and its W12-1067 wild-type, differences in the normalized ^{13}C-enrichments (Δmyo-Inositol mutant – WT) are shown by the numbers in **Figure 18B**. Higher fluxes in the Δmyo-inositol mutant are indicated by normal arrows, whereas lower fluxes are shown by dashed arrows. It was obvious that the Δmyo-inositol mutant metabolized glucose more efficiently into sugars, alanine and lactate. Using ^{13}C-glycerol as a precursor, also more flux from glycerol was observed into alanine and lactate in the mutant. It follows that more ^{13}C-flux from glucose or glycerol is directed into glycolysis and its downstream products due to the absence of enzymes putatively involved in myo-inositol metabolism.



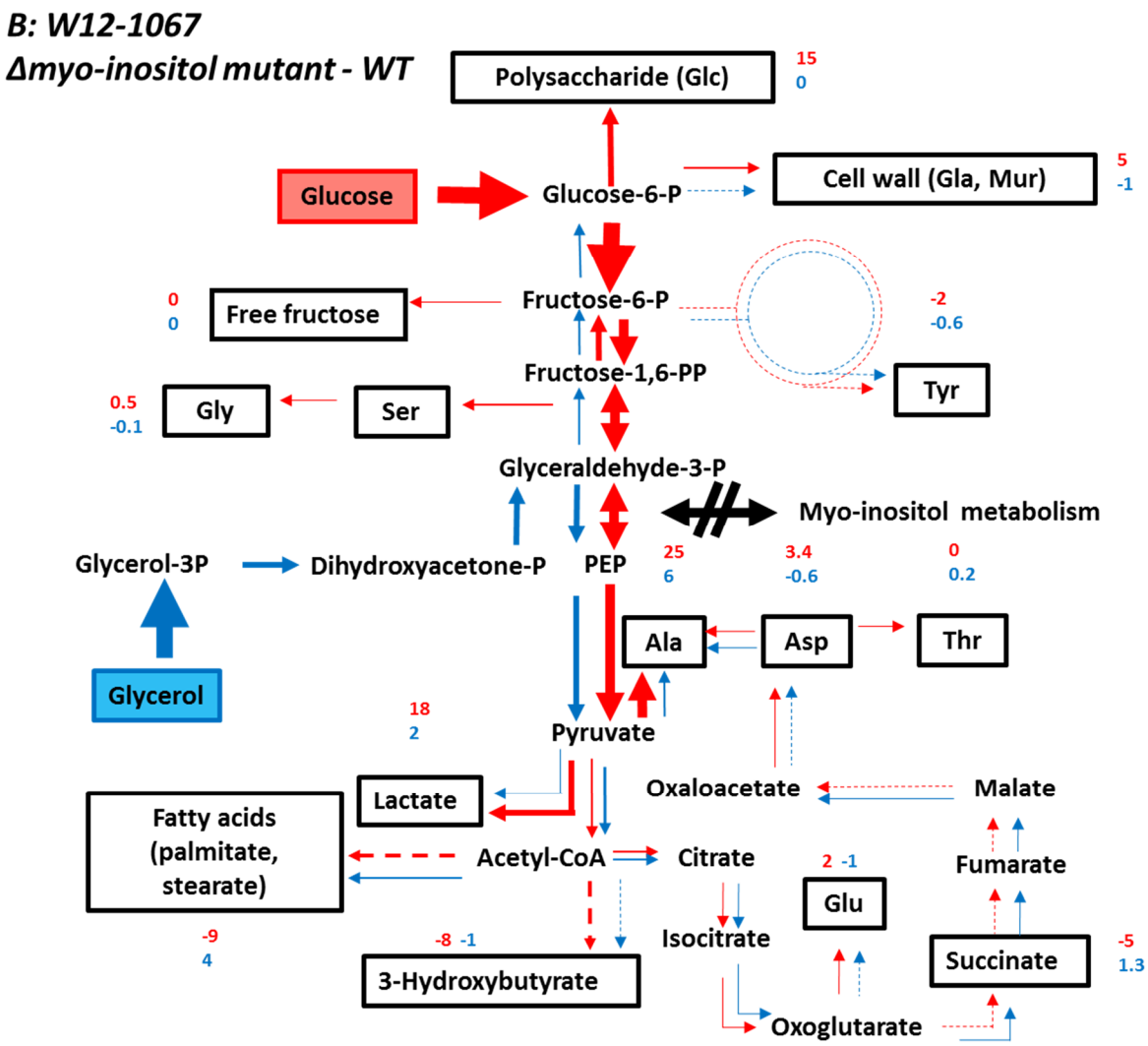


Figure 18. (A) Observed metabolic pathways and fluxes in *Francisella sp.* W12-1067 Δmyo-inositol mutant from exogenous glucose (red arrows), and glycerol (blue arrows). (B) Differences in the metabolic fluxes between *Francisella sp.* W12-1067 WT and Δmyo-inositol mutant. Metabolites studied by GC-MS-based isotopologue profiling are indicated by black boxes. The numbers indicate normalized overall ^{13}C-enrichments (from labelled glucose, glycerol and serine in red, blue and green, respectively). The arrow widths roughly indicate the relative fluxes. Fluxes conducive to enrichments <1% are not shown. In (B) the numbers indicate the differences in the normalized overall ^{13}C-enrichments (Δmyo-inositol mutant – WT). Higher fluxes in Δmyo-inositol mutant are indicated by normal arrows, lower fluxes by dashed arrows.

3.2.5 Analysis of myo-inositol metabolism of *Francisella* sp. W12-1067 grown in CDM medium

As shown in **Figure 3**, myo-inositol supports the growth of *F.* W12-1067, suggesting that myo-inositol is metabolised by this species. To provide further evidence for the pathway of myo-inositol metabolism in this strain, we then performed ^{2}H-labelling experiments with *F.* W12-1067 WT and its Δmyo-inositol mutant growing in CDM medium supplied with 9.13 mM myo-inositol-C-$^{2}H_6$.

The ^{2}H-enrichments and the fractional isotopolog distributions in sugars, protein derived amino acids, free amino acids and free metabolites were determined by GC/MS analysis (see **Figure 19**). High levels of ^{2}H was found in sugars, protein derived amino acids and free metabolites of up to 8% in *F.* W12-1067 wild-type. In sharp contrast, only very low ^{2}H-values were observed in the same metabolites from the Δmyo-inositol mutant (**Figure 19A**). This provides firm evidence for the uptake and utilization of myo-inositol by enzymes encoded in the myo-inositol operon described above. Consequently, lack of these enzymes in the mutant resulted in no or very low levels of ^{2}H-incorporation in the mutant strain.

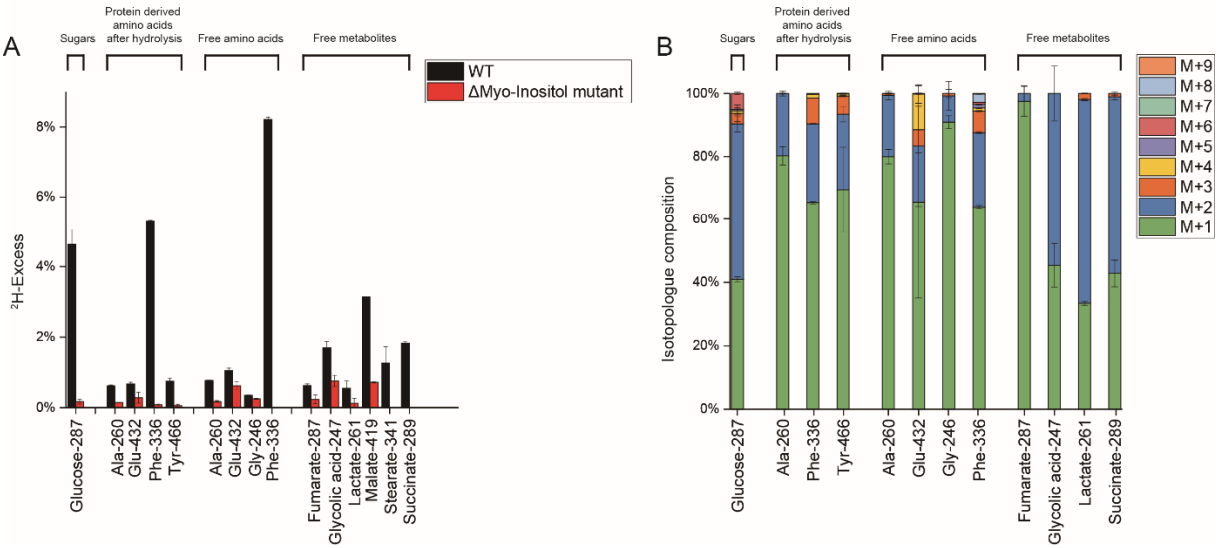


Figure 19. D-Excess (mol%) and **(B)** the fractional isotopolog distributions (%) in key metabolites of *Francisella* W12-1067 WT strain and *Francisella* W12-1067 Δmyo-inositol mutant grown in CDM medium supplied with 9.13 mM *myo*-inositol-C-d$_6$. D-Excess (mol%) and relative fractions of isotopologs (%) were determined by GC/MS of silylated derivatives at the indexed m/z values. Error bars indicate standard deviations from the means of the 3 × technical replicates values. M+1, M+2, M+3, etc. indicate isotopologs carrying 1, 2, 3, etc. ^{2}H-atoms. For numerical values, see **Supplemental Tables S90-95**.

Isotopologue profiling also revealed some insight into the mechanisms of myo-inositol usage. Glucose acquired one to two ^{2}H-atoms from the $^{2}H_6$-labelled MI tracer at relative amounts of 40 and 50 %, respectively (**Figure 19B**). This pattern suggest that $^{2}H_6$-myo-inositol is converted into M+3 isotopologs of 2-dehydro-3-deoxy-D-gluconate serving as a precursor for M+1 isotopologs in pyruvate and M+2 isotopologs of glyceraldehyde-3-P after cleavage by the Eda enzyme (**Figure 20**). These precursors are both derived from M+4 labeled 2-dehydro-3-deoxy-D-gluconate, although the synthesis of pyruvate leads to a loss of one deuterium atom (hence the M+1 labelling), whereas the formation of glyceraldehyde 3-phosphate keeps both deuterium atoms (hence the M+2 labelling). Either precursor enters the glycolysis leading to M+1 and M+2 isotpologs of glucose, respectively.

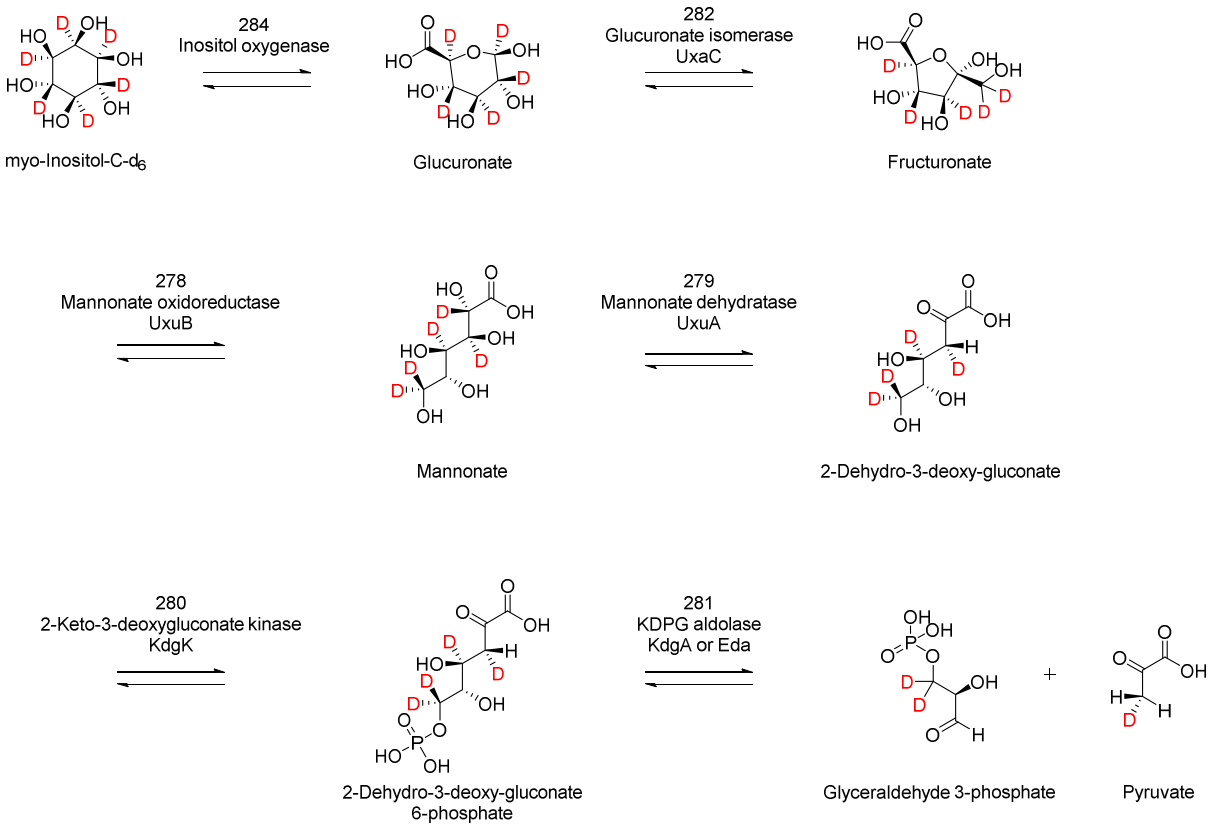


Figure 20. Myo-inositol-C-d_6 degradation pathway

After the hydrolysis of the bacterial cell, protein derived amino acids could be obtained. GC/MS analysis of 15 TBDMS-amino acids revealed ^{2}H-enrichments in 5 amino acids: Phe > His > Tyr > Glu > Ala (5.3-0.7%) (**Figure 19A**). Because of the unlabelled amino acids present in the CDM medium, the other amino acids were apparently unlabelled. The M+1 isotopologs in pyruvate and M+2 isotopologs of glyceraldehyde 3-phosphate resulted in the M+1 and M+2 isotopologs in alanine. About 80% of alanine was M+1 labelled, which suggested the direct formation of $^{2}H_1$-Ala from $^{2}H_1$-pyruvate. Phenylalanine and tyrosine were more complex and contained a large fraction of M+1 and a less fraction of M+2, which could be reflect the formation of shikimate/chorismate from labelled pyruvate and erythrose 4-phosphate via the PPP. Surprisingly and for unknown reason, His was highly labelled (2.1%, **Supplemental Table S90**), and contained mainly the M+1 isotopolog.

Generally, the ^{13}C-enrichments and isotopolog profiles of free amino acids resembled those from the protein-bound amino acids (**Figure 19A**). As well as alanine, lactate was M+1 and M+2 labelled, with M+2 being the major isotopolog (**Figure 19B**). It was speculated that alanine and lactate were formed during different growth phases, which lead to the different isotopolog distribution. Succinate and fumarate were formed from the $^{2}H_1$-pyruvate and $^{2}H_2$-glyceraldehyde 3-phosphate via

$^2H_1/^2H_2$-oxaloacetate, which explained the M+1 and M+2 isotopologs in succinate and the M+1 isotopologs in fumarate.

3.2.6 Discussion

Some bacteria are able to use myo-inositol (cyclohexane-1,2,3,4,5,6-hexol; $C_6H_{12}O_6$) as the (sole) carbon source (Yoshida et al., 2008). One of the myo-inositol pathway (Iol operon) uses a myo-inositol dehydrogenase (IolG) as the first step to convert myo-inositol to DHAP and acetyl-CoA (Anderson and Magasanik 1971). A second pathway (glucuronate [myo-inositol] metabolism pathway) use a myo-inositol oxygenase as the first step to convert myo-inositol to pyruvate and 3-PG (Peekhaus and Conway, 1998; Ashwell et al., 1960, Kilgore and Starr 1959). Myo-inositol is a membrane component and a secondary messenger in eucaryotic cells. In humans, myo-inositol is synthesized from glucose-6-phosphate. In addition, bacteria, fungal and plants are also able to generate myo-inositol from phytate by the enzymatic activity of phytases (myo-inositol hexakisphosphate phosphohydrolase) (Rao et al., 2009). Phytate is an important phosphate and carbon storage of plants and is found at high amounts in fruits, nuts seeds, and therefore also in soil and aquatic habitats (Lim et al., 2007). This is in agreement with the presence of a myo-inositol-utilizing pathway in *Francisella* strains associated with aquatic habitats (see below).

Rydzewski and her co-workers recently identified a new *Francisella* species (water-associated environmental isolate *F*. W12-1067) (Rydzewski et al., 2014) and in this work the *in silico* genome analysis revealed the presence of a putative glucuronate metabolism pathway in this species. The characteristic myo-inositol oxygenase of *F*. W12-1067 is 81-88% identical to the respective enzymes of different *Francisella* species and 41% identical to the respective oxygenase of the plant *Arabidopsis lyrata*. We could demonstrate that in *F*. W12-1067 the main glucuronic-utilization pathway is present (**Figure 3**).

It was demonstrated that myo-inositol supports growth of *F*. W12-1067 in CDM, suggesting the utilization of myo-inositol by these species, but not of the myo-inositol *F*. W12-1067 mutant. The utilization of myo-inositol by *F*. W12-1067 could then be demonstrated by the usage of CDM and myo-inositol-C-d_6 as the growth substrate (**Figure 21**). Whereas the wild-type strain showed high utilization of myo-inositol, only less flux of ^2H from myo-inositol into the mutant strain was detectable. Less amounts of myo-inositol may enter the cell of the mutant strain although the myo-inositol transport protein (peg283) is lacking, due to the presece of a minor myo-inositol transporter (peg112, Pfam MFS_1) or the putative sugar permease (peg717, Pfam MFS_1) in *F*. W12-1067. The ^2H-enrichment indicates that myo-inositol-C-d_6 serves as an efficient carbon substrate leading to great

variety of isotopologue composition of sugars, protein derived amino acids, free metabolites and free amino acids. As pointed out above, (synthesized) glucose is derived from the M+1 labeled pyruvate and M+2 labeled glyceraldehyde 3-phosphate generated by the Eda enzyme (peg 281), known from the Entner-Doudoroff pathway and also present at the myo-inositol-utilizing operon of *F*. W12-1067.

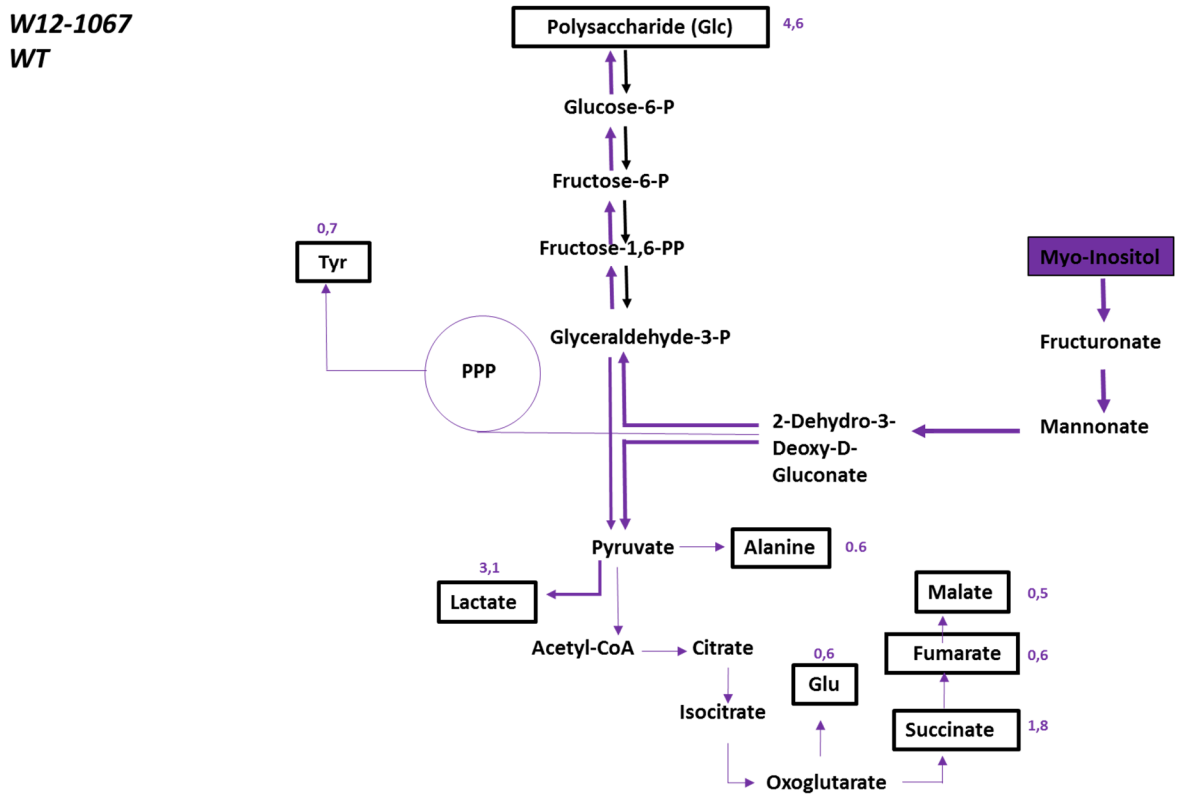


Figure 21. Observed metabolic pathway and flux in F. W12-1067 from myo-inositol. The numbers present the ^{13}C-excesses and the arrow widths indicate the relative fluxes.

In addition to the utilization of myo-inositol, we were also interested in the metabolic capacity of the new species for its further characterization. Therefore, we investigate also the metabolism of main substrates (glucose, serine and glycerol) of this species. *F*. W12-1067 could well metabolize glucose, serine and glycerol into sugars, protein derived amino acids, free amino acids and free metabolites. Glucose is the most efficient precursor, which is degraded via EMP not the ED or PPP into glycogen, cell wall amino sugars amino acids and free fatty acids. However, the carbon flux from serine and glycerol was less active, which converted mostly into amino acids (Alanine), lactate and fatty acids when grown in the presence of unlabelled glucose (medium T). The substrate serine is mainly used directly for protein biosynthesis or for biosynthesis of Ala >Gly >FA >Glu, and serine was not effectively used for gluconeogenesis. It is indicated that *F*. W12-1067 utilized the additional carbon nutrient following the concepts of a bipartite metabolism. The Δmyo-inositol mutant of *F*. W12-1067

which is missing the whole MI-utilizing pathway, had a more active EMP pathway and less flux to the PPP, that indicated by higher labelling rates of alanine, lactate and sugars. It is suggested the degradation of glucose and glycerol via myo-inositol pathway is active. Interestingly, in medium T, the metabolism of glucose and glycerol in the mutant strain leads to higher carbon flux to Ala (pyruvate) and lower flux to Tyr, indicating that the Eda enzyme in the wild-type strain may not only be involved in the metabolism of myo-inositol. The absence of Eda may lead to a higher concentration of pyruvate and glyceraldehyde 3-phosphate, since there is no carbon flux to KDPG.

In conclusion, our findings confirm the *in silico* genome data of *F.* W12-1067, myo-inositol could be well metabolized as a nutrient. Moreover, our findings are also in agreement with multiple substrate usage by *F.* W12-1067 carbon fluxes from exogenous glucose, serine and glycerol. The bipartite metabolism of *F.* W12-1067 could be an argument for its host-specific virulence study. Furthermore, with the labelling experiments data of Δmyo-inositol mutant it is tempting to speculate that that the Eda enzyme in the wild-type strain may not only be involved in the metabolism of myo-inositol, further study of myo-inositol metabolism of *F.* W12-1067 could elucidate.

3.3 Differential carbon fluxes in *Bacillus subtilis* growing in biofilm or planktonic states

3.3.1 Experimental approach

In this work, to analyse the metabolism of different *B. subtilis* strains in planktonic or on agar plate, [U-$^{13}C_6$]glucose or [U-$^{13}C_5$]glutamate were used as ^{13}C tracers. Specifically, three different *B. subtilis* mutants were investigated in this work, the undomesticated strain NCIB 3610, the Δ*ymdB* derivate of NCIB3610 (strain GP921), which is defective in biofilm formation and the Δ*sinR* derivate of NCIB3610 (strain GP1562), which is directed to grow as biofilms. Each mutant was grown in CSE medium supplemented with 13.4 mM [U-$^{13}C_6$]glucose and on agar plate with 9 mM [U-$^{13}C_6$]glucose or 5.4 mM [U-$^{13}C_5$]glutmate, respectively. Using physical or chemical procedures, different sugars from the cell wall or glycogen, protein derived or free amino acids, and free polar metabolites were isolated from the dried cell pellets. Each of the metabolites was converted into nonpolar derivatives which were later analysed by GC/MS spectrometry (for details, see **Materials and Methods**). The labelling experiments were repeated at least once (biological replicates), and each sample was analysed three times by GC/MS spectrometry (technical replicates). Based on the biological and technical replicates, six experimental values were detected for each metabolite. Mean values and standard deviations were then calculated on this basis (see **Supplemental Tables S52-69** for numerical data).

3.3.2 ^{13}C-Labelling experiments in liquid cultures

To analyse the metabolism of the strains growing in liquid culture, labelling experiments were performed in liquid CSE medium containing 13.4 mM [U-$^{13}C_6$]glucose and 13.9 mM unlabelled glucose (**Supplement Table S72**). Each of the labelling experiments was repeated once (biological replicate). The strains were grown at 28 °C until a final optical density (OD_{600}) of approximately 1.0. The bacteria were pelleted, frozen and lyophilized affording about 100 mg of dry cell pellet from a given experiment.

From the dried cell pellets, sugars, amino acids and free metabolites were extracted and converted into volatile derivatives for GC-MS analysis as described above. Each sample was analysed three times in consecutive runs (technical replicates). Based on the biological and technical replicates, at least six experimental values of each metabolite were obtained resulting in mean values and standard deviations given below (See also **Supplemental Tables S52-S54** and **S61-S63** for numerical data).

3.3.2.1 ^{13}C-Excess and isotopologue profiles of sugars

Glucose, glucosamine and muramic acid were obtained from hydrolysates of the three different *B. subtilis* strains under study. GC-MS analysis afforded highly similar ^{13}C-enrichments (about 40%, **Figure 22**, **Supplement Table S54**) and isotope distributions (**Figure 23**) of the sugars when comparing the three strains. Since the molar ratio of labelled and unlabelled glucose in the CSE medium was about 1:1, it can be concluded that the major fraction (80 – 85 %) of the analysed sugars was formed from the glucose supplement in the medium. In other words, only a minor fraction was derived by gluconeogenesis using unlabelled carbon sources present in the medium (i.e. glutamate, citrate, or succinate). The major isotopologue (80 – 40%) of the analysed sugars carried six ^{13}C-atoms (denoted as M+6 due to a mass characterized by six mass units on top to the mass M of the unlabelled derivative, i.e. carrying six ^{13}C-atoms). Obviously, these isotopologues were directly derived from the [U-$^{13}C_6$]glucose supplement. However, M+1 to M+5 isotopologues were also observed in muramic acid (in total about 60%), glucosamine (in total about 40%) and glucose (in total about 20%) indicating some glycolytic cycling involving labelled and unlabelled fragments in rebuilding hexoses prior to the incorporation into the respective polysaccharides (muramic acid > glucosamine > glucose).

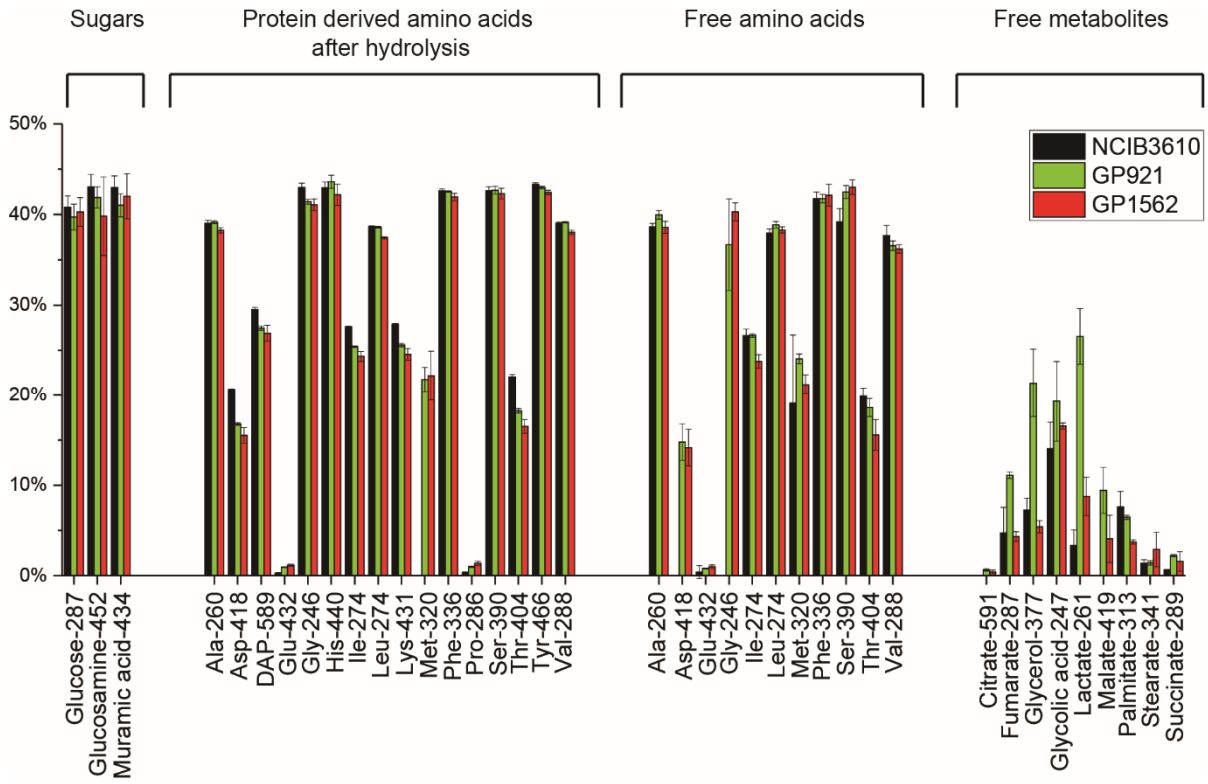


Figure 22. ^{13}C-Excess (mol%) in key metabolites of Bacillus subtilis subspecies NCIB 3610 (black), GP921 (green) or GP1562 (red) grown in CSE medium supplied with 13.4 mM [U-$^{13}C_6$]glucose. ^{13}C-Excess (mol%) were determined by GC/MS of silylated or esterized derivatives at the indexed m/z values. Error bars indicate standard deviations from the means of 6 values 2 × biological replicates, 3 × technical replicates. For numerical values, see **Supplemental Tables S52-S54**.

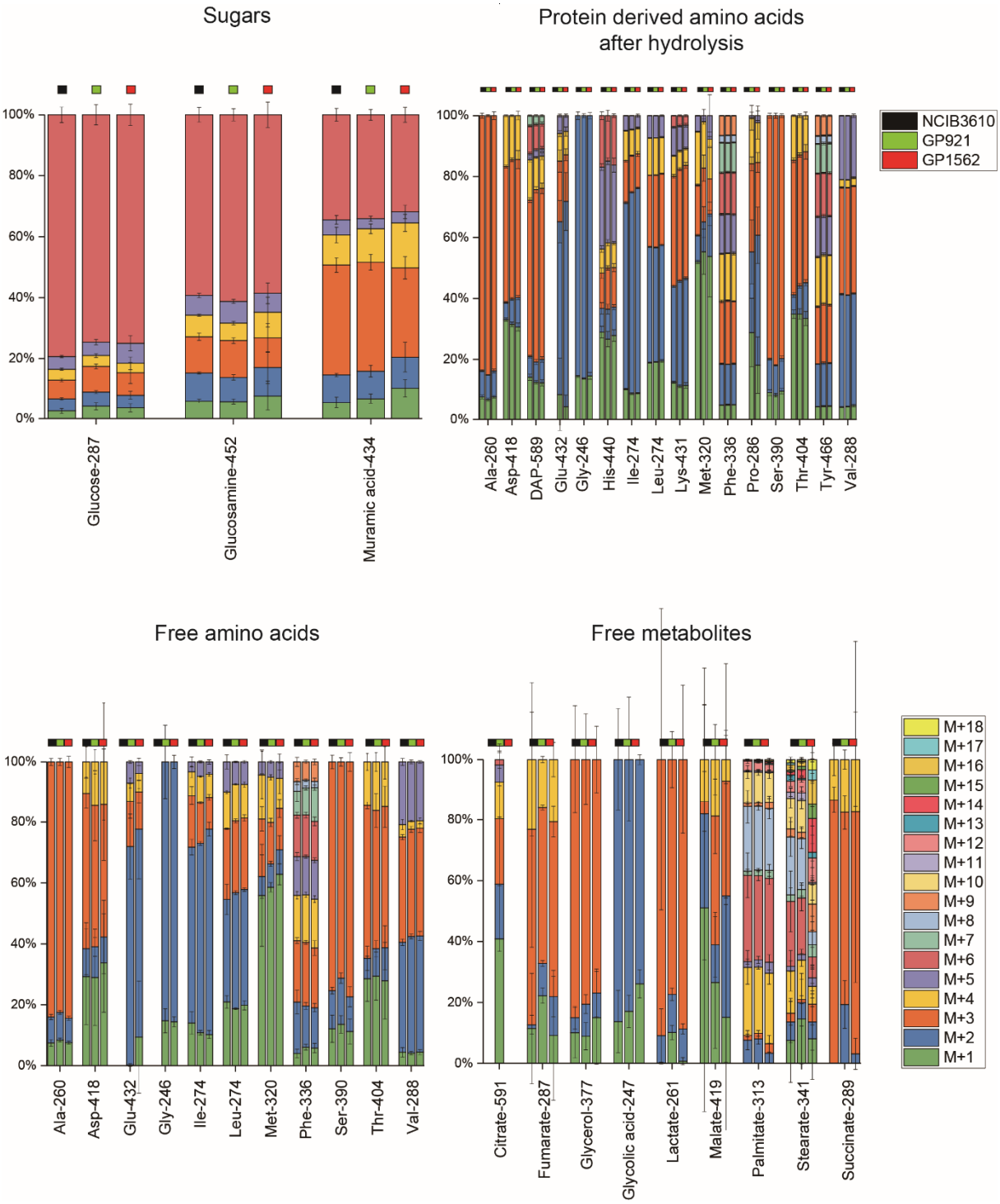


Figure 23. The fractional isotopolog distributions (%) in key metabolites of *B. subtilis* subspecies NCIB 3610 (black), GP921 (green) or GP1562 (red) grown in CSE medium supplied with 13.4 mM [U-$^{13}C_6$]glucose. Relative fractions of isotopologs (%) were determined by GC/MS of silylated or esterized derivatives at the indexed m/z values. Error bars indicate standard deviations from the means of 6 values 2 × biological replicates, 3 × technical replicates. M+1, M+2, M+3, etc. indicate isotopologs carrying 1, 2, 3, etc. ^{13}C-atoms. For numerical values, see **Supplemental Tables S61-S63**.

3.3.2.2 ^{13}C-Excess and isotopologue profiles of protein or cell wall-derived amino acids

Acidic hydrolysis of the cells afforded a mixture of amino acids (mostly derived from the protein and cell wall fractions) which were converted into TBDMS-derivatives for GC/MS analysis. In general, amino acids displayed a broad range of ^{13}C enrichments in the order: Tyr > Gly > His > Ser > Phe > Ala > Val > Leu > DAP > Lys > Ile > Thr > Asp > Met > Pro > Glu (43-0.2%) (**Figure 22** and **Supplemental Table S52**). More specifically, a set of amino acids carried the ^{13}C-label at about 40 % (i.e. Tyr, Gly, His, Ser, Phe, Ala, Val, Leu) reflecting their predominant origin from the glucose supplement in the medium. Notably, these amino acids were derived from intermediates of the pentose phosphate pathway (i.e. His from 5-phosphoribosyl pyrophosphate and Tyr from erythrose 4-phaosphate) or the glycolytic pathway (i.e. Ser and Gly from phosphoglycerate, Ala, Val and Leu from pyruvate). Again, gluconeogentic sources did not significantly (i.e. about > 15 %, **Figure 23**) contribute to their biosynthesis. However, the ^{13}C enrichments for Glu and the related amino acid Pro were < 2 % reflecting high rates of incorporation of unlabelled glutamate from the medium and its usage for bacterial Glu and Pro biosynthesis. Amino acids derived from oxaloacetate, i.e. Asp, Thr, Lys, Met, Ile and diaminopimelate (DAP) were also relatively low (< 15 - 30%) showing carbon fluxes from the labelled glucose tracer and the unlabelled supplements in the medium (e.g. succinate and glutamate). Comparing the overall ^{13}C enrichments of Asp, DAP, Ile, Lys and Thr in the three different mutants, small but significant differences were found (NCIB3610 > GP921 > GP1562) (**Figure 22**). This pattern could point at a more active TCA cycle in GP921 and GP1562 shuffling more unlabelled glutamate via α-ketoglutarate into oxaloacetate and its down-stream products, Asp, Thr, DAP, Lys and Ile. However, the isotopologue compositions of all amino acids did not significantly differ in the different strains (**Figure 23**) suggesting that the pathways from glucose into these amino acids were highly similar if not identical when the stains grew in liquid medium.

Nevertheless, in conjunction with the specific ^{13}C enrichments described above, the unique profiles could also be used to reconstruct the metabolic pathways and relative fluxes from glucose in *B. subtilis*. The isotopologue distribution of Ala mainly showed a M+3 species (80-85%) representing its precursor [U-$^{13}C_3$]pyruvate derived from [U-$^{13}C_6$]glucose by glycolysis. Asp comprised relatively high fractions of M+1 and M+3 isotopologues (**Figure 23**) indicating their formation via oxaloacetate made by carboxylation of pyruvate and/or PEP. M+2 and M+4 species in Asp can be explained by M+2 and M+4 in oxaloacetate via the TCA. The same profile was observed in Thr suggesting that Thr was formed from Asp via aspartate 4-semialdehyde, which also resulted in a similar ^{13}C composition in Ile. DAP and Lys had also similar isotopologue profiles, reflecting the formation of Lys after the decarboxlation of DAP using diaminopimelate decarboxylase. Surprisingly, the isotope distributions of Val and Leu were not similar, which could indicate that the formation of α-isopropylmalate from 3-methyl-2-oxobutanoic acid was not a majority pathway. The high fractions of M+1 and M+2 and

low fractions of M+3, M+4 and M+5 in Glu suggested a high turnover of TCA intermediates between oxaloacetate and α-ketoglutarate. After the dehydrogenation and oxidation of Glu via 1-pyrroline-5-carboxylate, proline is formed, as shown by its similar isotope distributions. The high fractions of [U-$^{13}C_3$]Ser (M+3) in the fragment containing all three carbon atoms of the original serine molecule (Ser-390) indicated efficient usage of glucose present in the CSE medium and the formation of [U-$^{13}C_3$]3-phosphoglycerate by degradation of [U-$^{13}C_6$]glucose. Gly is formed by serine hydroxymethyltransferase, as displayed by the high M+2 fraction in Gly. After degradation of glucose via the pentose phosphate cycle (PPP), histidine is formed form PRPP carrying M+1 to M+5 isotopologues. The labelling profiles of Phe and Tyr were more complicated and contained M+1 to M+9 fractions. That could be explained by the formation of erythrose 4-phosphate via the PPP and PEP via glycolysis, which were both degradation products of [U-$^{13}C_6$]glucose. Based on these observations, the metabolic network for *B. subtilis* can be reconstructed which basically confirms the models published previously (Dauner et al, 2000; Tännler et al.2008; Kohlsted et al., 2014). Further support for this network could be provided by the labelling pattern reported below for free amino acids and other polar metabolites.

3.3.2.3 ^{13}C-Excess and isotopologue profiles of free polar metabolites

More than 20 polar metabolites including free amino acids, lactate, glycerol, succinate, malate, and some fatty acids were extracted in amounts sufficient for isotopologue profiling. The ^{13}C-enrichments and isotope distributions of free amino acids (**Figure 22** and **23**) resembled those described for protein-derived amino acids. The GP921 mutant had the highest ^{13}C-excess values of lactate and glycerol. However, differences in the isotopologue distributions of lactate, glycerol and TCA intermediates from the different mutants were less evident (**Figure 23**). The high M+3 fractions of lactate indicated its origin from glycolytic [U-$^{13}C_3$]pyruvate. Glycerol had also abundant M+3, indicating high fractions of M+3 in glyceraldehyde phosphate, an intermediate of glycolysis. Notably, however, a gene encoding a glycerol dehydrogenase converting glyceraldehyde phosphate into glycerol was not assigned in the *B. subtilis* genomes (Mäder et al., 2011). The ^{13}C-enrichments and isotopologue profiles of TCA intermediates and free Asp again suggested that NCIB3610 does not have an active TCA metabolism to convert oxaloacetate into the TCA intermediates malate and fumarate. On the basis of the lower ^{13}C-enrichments in TCA intermediates, especially of fumarate from the GP1562 strain, this strain seems to be more active in succinate or glutamate uptake in comparison to GP921.

Fatty acids were also less labelled; their ^{13}C-enrichments were only 1–8% (**Figure 22, Supplemental Table S53**). The isotopologue profiles (**Figure 23**) showed high relative amounts M+2, M+4 etc.

reflecting their formation from [U-$^{13}C_2$]acetyl-CoA units. In line with this finding was the observation of M+2 isotopologues in succinate and fumarate derived from [U-$^{13}C_2$]acetyl-CoA via the TCA.

In total, the well-known core metabolism of *B. subtilis* (Mäder et al., 2011) is nicely reflected in these labelling data. The observed high ^{13}C excess values in sugars derived from bacterial polysaccharides indicated efficient uptake of exogenous glucose and its direct usage for cell wall and glycogen biosynthesis. However, the ^{13}C enrichments and specific isotopologue compositions in down-stream metabolic products also provided strong evidence for glucose degradation via glycolysis and the PPP. Due to the huge amounts of potassium glutamate and sodium succinate in the used CSE medium, the ^{13}C labelling of TCA intermediates and fatty acids was less efficient.

3.3.3 Labelling experiments of *B. subtilis* growing on agar containing [U-$^{13}C_6$]glucose

Since the labelling patterns obtained from the experiments with the *B. subtilils* strains grown under planktonic conditions did not reveal a major reprogramming of metabolite fluxes due to the genetic background, we performed additional experiments with the same strains growing on agar plate, where distinct phenotypes could be observed for the strains. For this purpose, the three different *B. subtilis* strains were first pre-cultured in LB medium, then dropped on MSgg agar plates containing 9.3 mM [U-$^{13}C_6$]glucose in addition to 18.5 mM unlabelled glucose, 24.6 mM unlabelled glutamate and 68 mM unlabelled glycerol (see also **Supplemental Table S74**).

In each of the labelling experiments, the strains were grown at 30°C for 72 h. The cells were then scratched off the surface and the cellular material of three macrocolonies were pooled for harvesting. The bacteria were pelleted, autoclaved and lyophilized affording about 10 mg of dry cell pellet from a given experiment. Each of the labelling experiments was repeated five times (affording six independent biological replicates), and each sample was analysed three times in GC/MS spectrometry (three technical replicates for each metabolite).

Using the procedures described above, we again extracted sugars, amino acids and free metabolites from the different strains. ^{13}C Excess and isotopologue compositions were assessed by GC/MS spectrometry. Based on the biological replicates and technical replicates, eighteen experimental values were obtained for each metabolite providing a robust calculation of mean values and standard deviations (see also S**upplemental Tables S55–S57** and **S64-S66** for numerical data). The high number of individual values also allowed statistical validation using the Excel Student's T-test affording p-values shown in **Supplemental Table S70**.

3.3.3.1 ^{13}C-Excess and isotopologue profiles of sugars

After hydrolysis of the cell pellet, polysaccharide derived glucose and the cell wall derived amino sugars, glucosamine and muramic acid, were isolated and analysed. In sharp contrast to the corresponding data from the same strains grown under planktonic conditions, significant differences of ^{13}C enrichments could now be found between the three strains. The GP1562 strain, the Δ*sinR* derivate of NCIB3610, which controls the biofilm formation, had the highest ^{13}C-enrichments (about 35-40%) (**Figure 24,** S**upplemental Tables S57**). This equals the maximum amount of ^{13}C which can be transferred from labelled glucose diluted with unlabelled glucose at an approximate ratio of 1:2 (**Supplemental Table S74**). Thus, in the mutant growing as a biofilm, the glycogen and cell wall derived sugars were almost completely (> 90 %, **Figure 25**) derived from the exogenous glucose. The other carbon sources in the medium (i.e. unlabelled glutamate and glycerol) did not play a major role in providing building units for gluconeogenesis under these conditions. In sharp contrast, the wild-type strain NCIB3610 and the GP921 strain (Δ*ymdB* derivate of NCIB3610 stalled to grow as a non-biofilm) had only ^{13}C-enrichments of 15-20% (**Figure 24**). This clearly indicates that another carbon source in the agar must have contributed to the sugar formation in these strains. Since exogenous glutamate did not play a significant role in this formation process (see below), it can be concluded that the (unlabelled) glycerol present in the medium must have been the carbon source for gluconeogenesis in the non-biofilm strains. The major isotopologue of all sugars was the M+6 species (**Figure 25**), again indicating efficient uptake and direct usage of glucose. However, the M+1 to M+5 fractions significantly contributed to the labelled molecules, especially in muramic acid (in total about 80%), and glucosamine (in total about 60-70%), but also in polysaccharide derived glucose (about 40-60%). Notably, these relative amounts of M+1to M+5 were slightly higher in the NCIB3610 strain confirming higher rates of glycolytic turnover via PPP or gluconeogenesis including glycerol as a source.

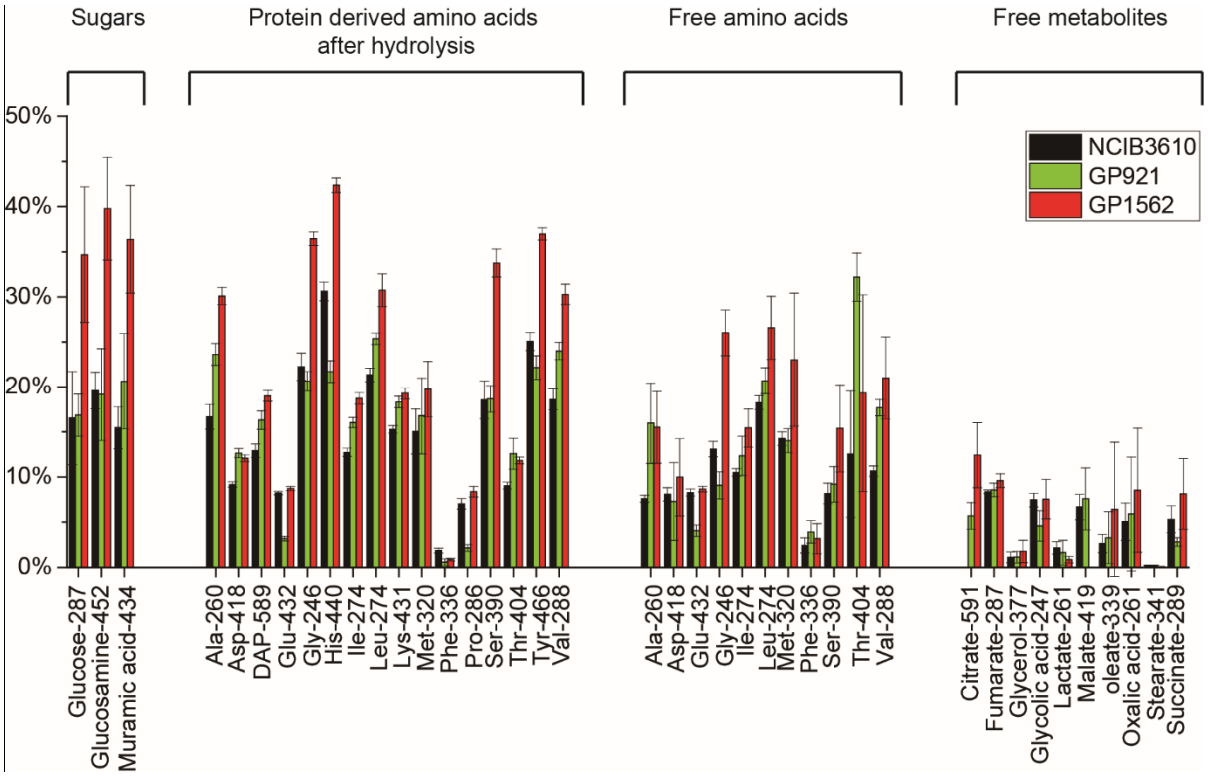


Figure 24. ^{13}C-Excess (mol%) in key metabolites of *B. subtilis* subspecies NCIB 3610 (black), GP921 (green) or GP1562 (red) grown on Msgg agar plate supplemented with 9 mM [U-$^{13}C_6$]glucose. ^{13}C-Excess (mol%) were determined by GC/MS of silylated or esterized derivatives at the indexed m/z values. Error bars indicate standard deviations from the means of 18 values 6 × biological replicates, 3 × technical replicates. For numerical values, see **Supplemental Tables S55-57**.

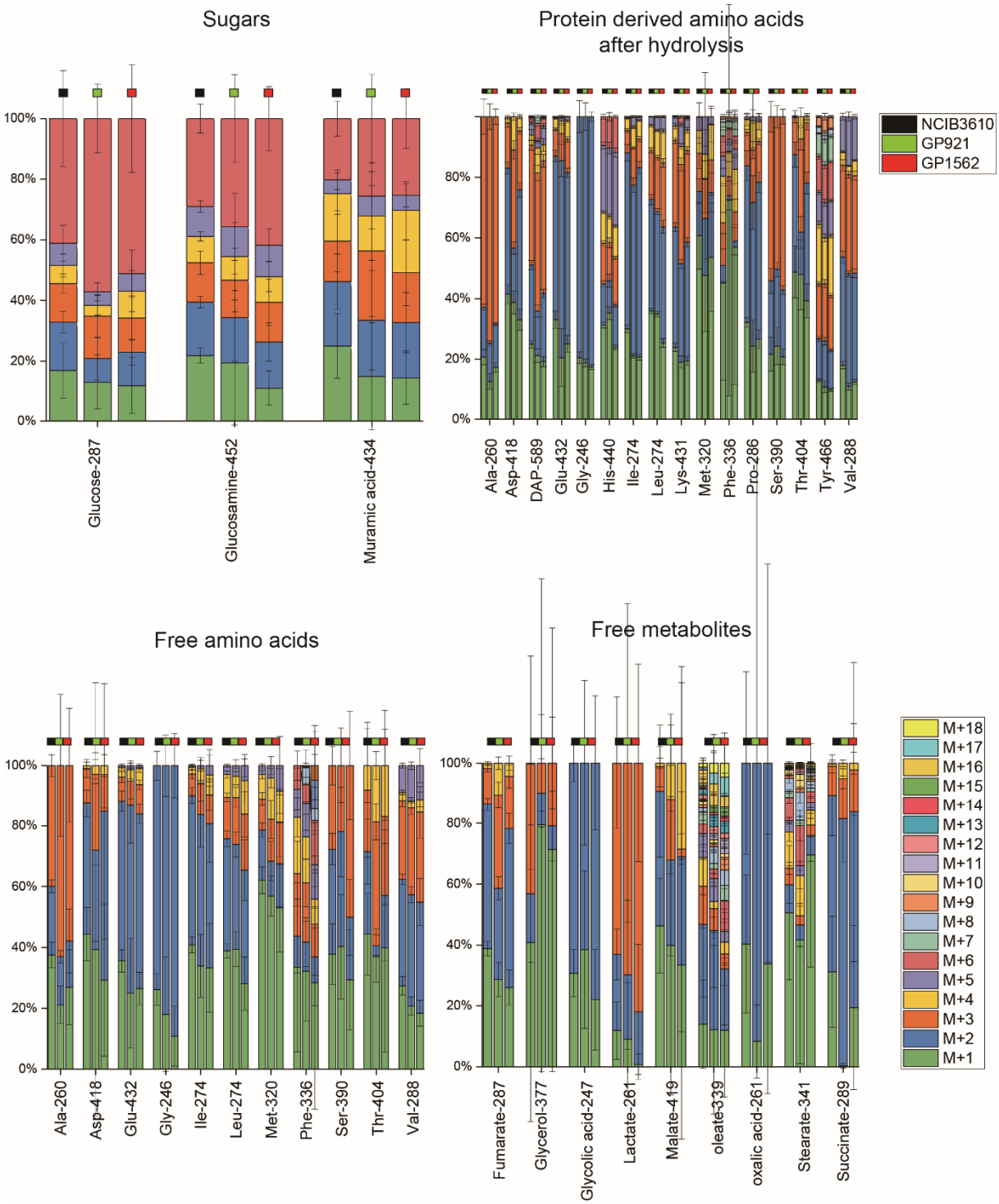


Figure 25. The fractional isotopolog distributions (%) in key metabolites of *B. subtilis* subspecies NCIB 3610 (black), GP921 (green) or GP1562 (red) grown on Msgg agar plate supplemented with 9 mM [U-$^{13}C_6$]glucose. Relative fractions of isotopologs (%) were determined by GC/MS of silylated or esterized derivatives at the indexed m/z values. Error bars indicate standard deviations from the means of 18 values 6 × biological replicates, 3 × technical replicates. M+1, M+2, M+3, etc. indicate isotopologs carrying 1, 2, 3, etc. ^{13}C-atoms. For numerical values, see **Supplemental Tables S64-66**.

3.3.3.2 ^{13}C-Excess and isotopologue profiles of protein or cell wall-derived amino acids

The bacterial cell pellet was hydrolysed under acidic conditions and resulting amino acids were converted into TBDMS derivatives for GC/MS analysis. Since the MSgg glucose / ^{13}C-glucose medium for the agar plate contained relatively high amounts of glutamate and phenylalanine (**Supplemental Tables S74**), the observed ^{13}C enrichments were low (< 10 %) for these amino acids and related ones (i.e. derived from glutamate such as proline) (**Figure 24** and **Supplemental Table S55**). Nevertheless, it is notable that despite the high amounts of glutamate in the medium, a considerable fraction of bacterial Glu/Pro acquired ^{13}C-label from exogenous glucose, especially in the NCIB3610 and GP1562 strains (about 8 %).

The highest incorporation rates (> 20%) were observed for amino acids related to their origin from intermediates of the PPP (i.e. His and Tyr) or glycolysis (i.e. Ser, Gly, Ala, Val and Leu) in line with our observations for the strains grown under liquid conditions. It is again notable that the ^{13}C values of these amino acids were highest (30 – 40 %) in the GP1562 strain indicating efficient usage of exogenous glucose for their respective biosynthesis via the PPP and glycolysis. The lower ^{13}C incorporation rates (15 – 30 %) into the same amino acids from the NCIB3610 and GP921 strains are explained by additional carbon fluxes starting from uptake and utilization of unlabelled glutamate and glycerol which were both present in considerable amounts in the agar medium. Because of the higher ^{13}C enrichment of glycolytic products e.g. of pyruvate in GP1562, higher ^{13}C enrichment of oxaloacetate and its downstream products Asp, DAP and Lys can be easily explained.

Comparing the ^{13}C enrichments of amino acids from the GP921 and NCIB3610 strains, GP921 displayed slightly higher ^{13}C enrichments in Ala, Ile, Leu and Val, which suggested more efficient degradation of glucose via glycolysis and PPP to pyruvate in GP921. Glu and Pro had lower ^{13}C enrichments in GP921 that could point at higher rates for the uptake of glutamate from the medium by GP921. However, Asp, DAP and Lys had higher ^{13}C enrichments in GP921 than in NCIB3610. It is therefore tempting to speculate that the formation of Asp via oxaloacetate from pyruvate is much more active in GP921 than in NCIB3610. The higher ^{13}C labelling in His and Tyr from the NCIB3610 indicate more flux via the PPP in NCIB3610.

No major differences were found in the isotopologue profiles between the three different strains (**Figure 25**), a clear indication that there were no significant differences between the general pathways forming amino acids from the labelled glucose in the strains under study. This finding was in line with the data for the bacteria growing under planktonic conditions.

3.3.3.3 ^{13}C-Excess and isotopologue profiles of free polar metabolites

Generally, ^{13}C excess values of free amino acids were lower than those of protein- or cell wall-derived amino acids (**Figure 24**), indicating that the glucose supply from the medium was diminished at the end of the growth (i.e. at the late logarithmic and stationary phase) when free amino acids were formed. The same conclusion can be made on the basis of the relatively low values for the free metabolites shown in **Figure 24**. However, the isotopologue distributions in the free amino acids were almost the same as in the protein- or cell wall- derived amino acids (**Figure 25**). This can be taken as evidence that the pathways from labelled glucose to the amino acids did not significantly change during the growth phases.

In total, after the labelling experiments with 9 mM [U-$^{13}C_6$]glucose in MSgg agar plate, unlike the planktonic samples, more significant differences were found between the three *B. subtilis* strains. The highest ^{13}C excess of different sugars explained that the GP1562 mutant converted most glucose into polysaccharide or amino sugars. NCIB3610 mutant had the most frequent glycolytic turnover via PPP or gluconeogenesis because of the lowest M+6 fraction. GP1562 had the most active glycolysis and PPP, and glycolysis was the main glucose degradation pathway. Asp and fumarate were mainly formed from pyruvate via oxaloacetate and the formation in GP921 was the most active in three strains. GP921 had the most efficient glutamate uptake. Compared to GP921, NCIB3610 has the more active glycolysis than GP921, but a more inactive PPP.

3.3.4 Labelling experiments of *B. subtilis* growing on agar containing [U-$^{13}C_5$]glutamate

To better identify the differential roles of other carbon substrates present in the medium, we now performed labelling experiments with the three different *B. subtilis* strains growing on MSgg agar plates containing 5.4 mM [U-$^{13}C_5$]glutamate in addition to 19.7 mM unlabelled glutamate and 68 mM unlabelled glycerol. Glucose was not present in this setting (**Supplemental Tables S74**). Again, the strains were grown at 30°C for 72 h on these plates and processed as described before for the experiment with labelled glucose. Each of the labelling experiments was repeated five times (affording six independent biological replicates), and each sample was analysed three times in GC/MS spectrometry (three technical replicates for each metabolite), again providing robust data for comparison.

3.3.4.1 ^{13}C-Excess and isotopologue profiles of sugars

The ^{13}C-enrichments of each sugar were less than 2.5% (**Figure 26**) indicating that the labelled glutamate supplement did not serve as a gluconeogenetic source. Rather, unlabelled glycerol present in the medium was used as a preferred carbon source for this purpose. Although low in the absolute values, the NCIB3610 strain displayed slightly higher ^{13}C-enrichments in comparison to the other strains indicating minor but significant rates of gluconeogenesis using carbon via the TCA (i.e. via oxaloacetate from glutamate-derived α-ketoglutarate). In agreement, the sugars were mostly M+1 and M+2 labelled (**Figure 27**) via M+1 and M+2 in oxaloacetate.

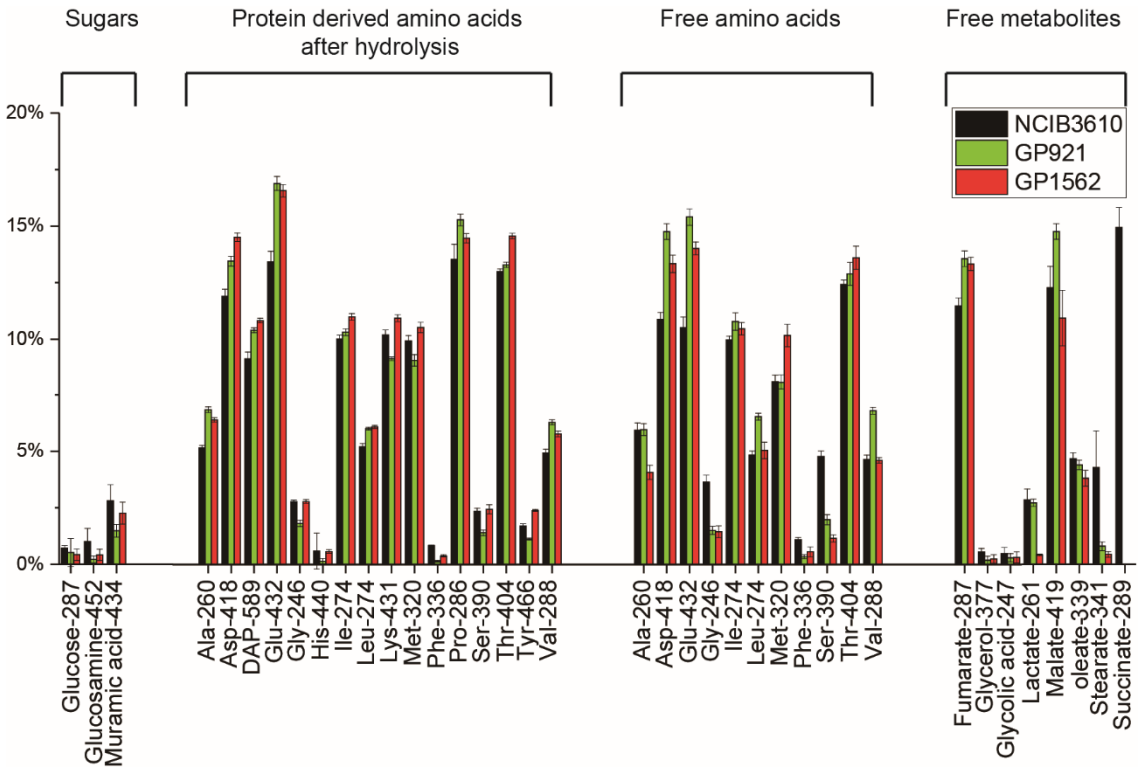


Figure 26. ^{13}C-Excess (mol%) in key metabolites of *B. subtilis* subspecies NCIB 3610 (black), GP921 (green) or GP1562 (red) grown on Msgg agar plate supplemented with 6 mM [U-$^{13}C_5$]glutamate. ^{13}C-Excess (mol%) were determined by GC/MS of silylated or esterized derivatives at the indexed m/z values. Error bars indicate standard deviations from the means of 9 values 3 × biological replicates, 3 × technical replicates. For numerical values, see **Supplemental Tables S58-60**.

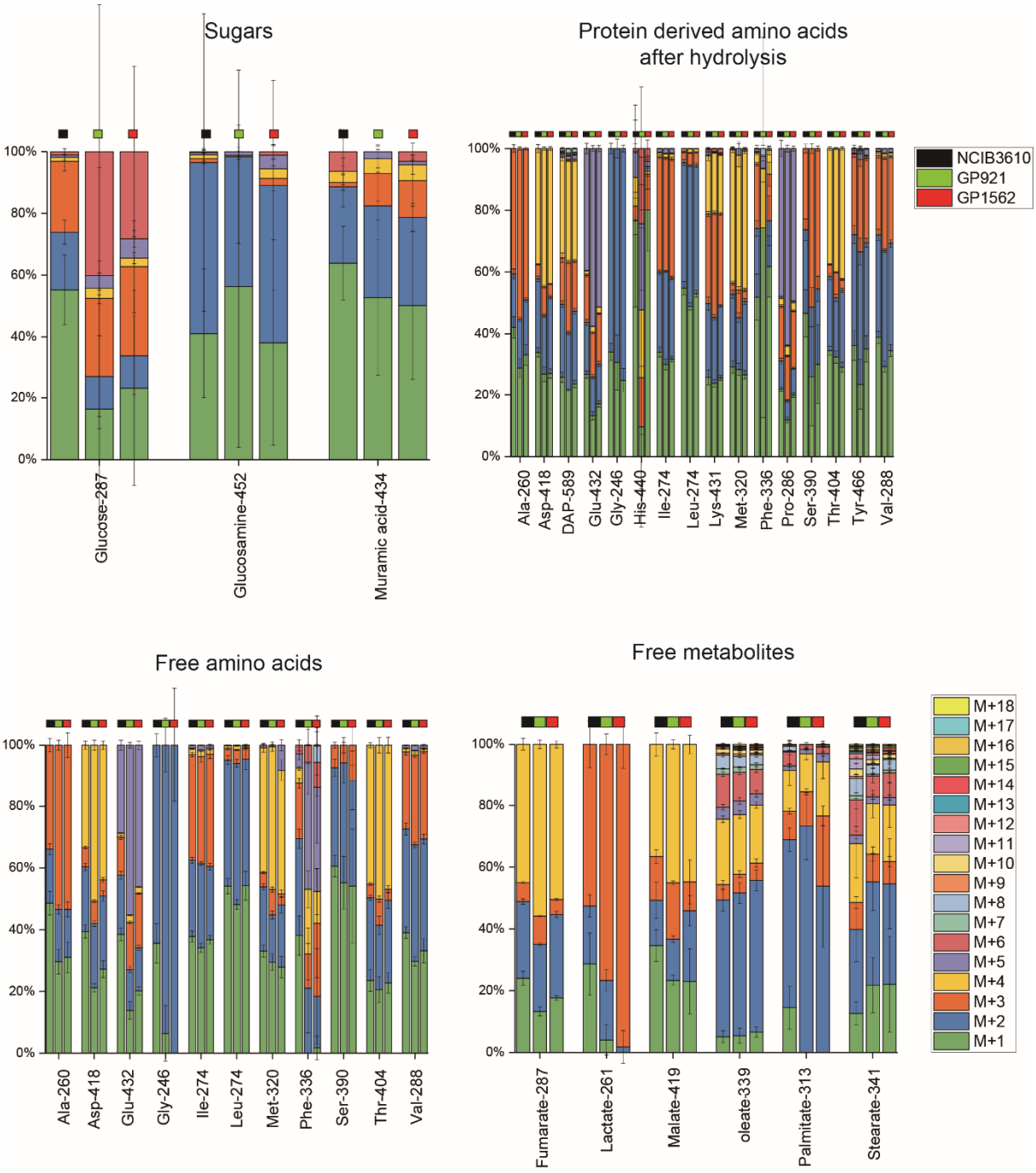


Figure 27. The fractional isotopolog distributions (%) in key metabolites of *B. subtilis* subspecies NCIB 3610 (black), GP921 (green) or GP1562 (red) grown on Msgg agar plate supplemented with 6 mM [U-$^{13}C_5$]glutamate. Relative fractions of isotopologs (%) were determined by GC/MS of silylated or esterized derivatives at the indexed m/z values. Error bars indicate standard deviations from the means of 9 values 3 × biological replicates, 3 × technical replicates. M+1, M+2, M+3, etc. indicate isotopologs carrying 1, 2, 3, etc. ^{13}C-atoms. For numerical values, see **Supplemental Tables S67-69**.

3.3.4.2 ^{13}C-Excess and isotopologue profiles of protein or cell wall-derived amino acids

In contrast to the low ^{13}C enrichments in sugars, some protein or cell wall derived amino acids acquired the ^{13}C label from glutamate at much higher rates. In particular, those amino acids (Glu, Pro,

Asp and Thr) derived from intermediates of the TCA cycle were labelled at 10 – 15 % ^{13}C (**Supplemental Tables S58**). Taking into account the dilution factor of exogenous ^{13}C-glutamate with unlabelled glutamate (1:4), it can be concluded that 50 – 75 % of Glu, Pro, Asp and Thr were *de novo* formed from the glutamate source in the medium. Lower values (6 – 1 % ^{13}C) were observed for amino acids derived via intermediates and products from the PPP or the glycolytic pathway. Here, glycerol was the preferred carbon source. Comparing the different strains, some minor differences could be found. In comparison to NCIB3610, Asp, DAP, Glu and Lys from GP1562 had higher ^{13}C enrichments (**Figure 26**), which suggested that the uptake of Glu and its conversion into oxaloacetate/Asp and its downstream products Lys and DAP via the TCA was more active in the biofilm strain GP1562. Similarly, higher fluxes from Glu to pyruvate via the TCA were also evident in GP1562 as well as in GP921 as reflected by the higher ^{13}C enrichments in Ala, Ile, Leu and Val.

Isotopologue profiles (**Figure 27**) resembled the expected ones following the routes of TCA cycling and pyruvate/PEP formation from oxaloacetate. Notably, no significant differences could be detected between the strains under studies.

3.3.4.3 Isotopolog Profiles of Free Polar Metabolites

No significant differences in the ^{13}C enrichments were found between the protein-derived amino acids and free amino acids. This indicates that exogenous glutamate was still present at the later growth phases. Because of the efficient shuffling of glutamate into the TCA, the ^{13}C-enrichments of fumarate, malate, and succinate (when observed in the GC runs) were high (10 -15 %). Free fatty acids isolated from GP1562 and GP921 strains were apparently unlabelled, however, interestingly for unknown reasen the free fatty acids isolated from NCIB3610 strain were about 5% labelled.

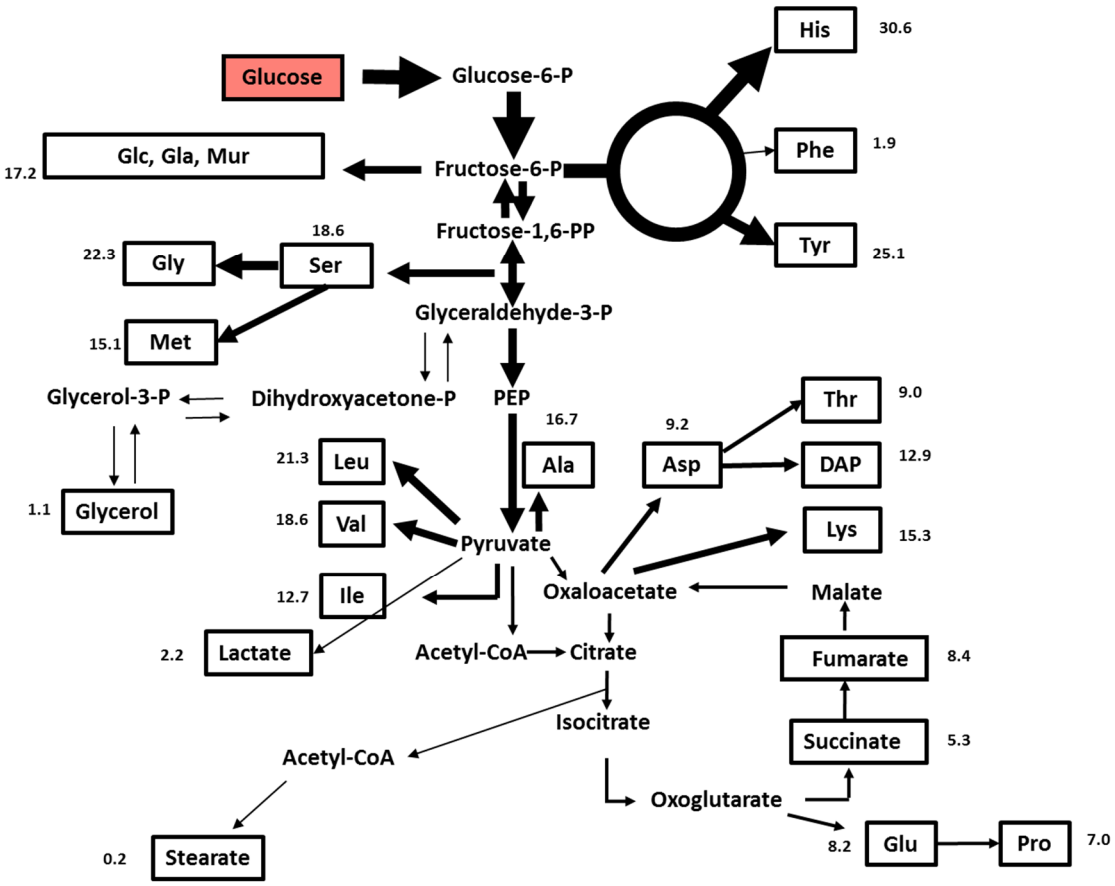
3.3.5 Discussion

The *B. subtilis* NCIB3610 wild-type strain and its Δ*sinR* and Δ*ymdB* mutant strains were grown under planktonic or biofilm conditions. ^{13}C-Labelling experiments using [U-$^{13}C_6$]glucose or [U-$^{13}C_5$]glutamate provided detailed information about the substrate usages, metabolic pathways and relative carbon fluxes for the specific strains and conditions. Generally, the well-known core metabolism of *B. subtilis* (Mäder et al., 2011) was nicely reflected by all of these labelling data. Starting from [U-$^{13}C_6$]glucose as a supplement to the liquid or agar medium, the observed high ^{13}C excess values in sugars derived from bacterial polysaccharides indicated efficient uptake of exogenous glucose and its direct usage for cell wall and glycogen biosynthesis (**Figure 28A**). Moreover, the ^{13}C enrichments and specific isotopologue compositions in down-stream metabolic products also provided

strong evidence for glucose degradation via glycolysis and the PPP, as well as of ^{13}C-fluxes via the TCA cycle and its anplerotic reactions. Due to the huge amounts of unlabelled carbon substrates in the respective media (i.e. glutamate, succinate and glycerol in the used CSE medium for liquid growth, and glutamate and glycerol in the used agar medium), the ^{13}C incorporation rates of downstream metabolite were specifically lowered due to fluxes involving these unlabelled substrates. In a complementary experimental setting using [U-$^{13}C_5$]glutamate as a supplement, the carbon fluxes via its deamination product, α-ketoglutarate, and subsequently via the TCA cycle could be quantified (**Figure 28B**). Whereas the labelling patterns in multiple metabolites from the strains growing under planktonic conditions were highly similar and therefore hardly revealed any differences in the pathways and fluxes used by the three strains. On this basis, it can be concluded that the deletion of the *sinR* and *ymdB* genes did not significantly change the substrate usages, pathways and core metabolic fluxes when grown in liquid medium.

In sharp contrast, however, significant differences were identified between the three *B. subtilis* strains when grown on agar. To better visualize these effects, **Figure 29** displays differences in the ^{13}C-enrichments and fluxes as numbers for key metabolites and arrow widths for differential carbon fluxes in the experiment with [U-$^{13}C_6$]glucose (**Figure 29A and B**) and [U-$^{13}C_5$]glutamate (**Figure 29C and D**). Specifically, differences in the enrichments and metabolic fluxes between the GP1562 biofilm strain (i.e. the Δ*sinR* mutant) and the NCIB3610 wild-type strain (GP1562-NCIB3610, red arrows) are shown in **Figure 29A and C**, whereas the differences between the GP921 strain (i.e. the Δ*ymdB* mutant) and the NCIB3610 wild-type strain (GP921-NCIB3610, green arrows) are shown in **Figure 29B and D**. Higher fluxes are indicated by normal arrows and lower fluxes are shown by dashed arrows in these Figures. This analysis shows that GP1562 had a more active polysaccharide formation, amino sugar formation and glucose degradation to form Asp, DAP and Lys via oxaloacetate than the other two mutants, NCIB3610 had more active glycolytic turnover than GP921 and GP1562, and GP921 had the most uptake of glutamate. It is therefore suggestive to speculate that under biofilm conditions exogenous glucose is most efficiently converted into polysaccharides. However, concomitantly higher flux rates via glycolysis and PPP to form of Ala, Asp, DAP, and Lys are stimulated. In agreement, the formation of complex biofilms depends on the supply of polysaccharides and amino acids as linkers between the sugar layers.

A


Glucose
Glucose-6-P
His
30.6
Phe
1.9
Tyr
25.1
17.2
Glc, Gla, Mur
Fructose-6-P
Fructose-1,6-PP
18.6
22.3
Gly
Ser
15.1
Met
Glyceraldehyde-3-P
Glycerol-3-P
Dihydroxyacetone-P
PEP
16.7
Thr
9.0
9.2
Asp
DAP
12.9
21.3
Leu
Ala
1.1
Glycerol
18.6
Val
Lys
15.3
Pyruvate
12.7
Ile
Oxaloacetate
Malate
2.2
Lactate
Acetyl-CoA
Citrate
Fumarate
8.4
Isocitrate
Succinate
5.3
Acetyl-CoA
Oxoglutarate
8.2
Glu
Pro
7.0
0.2
Stearate

B

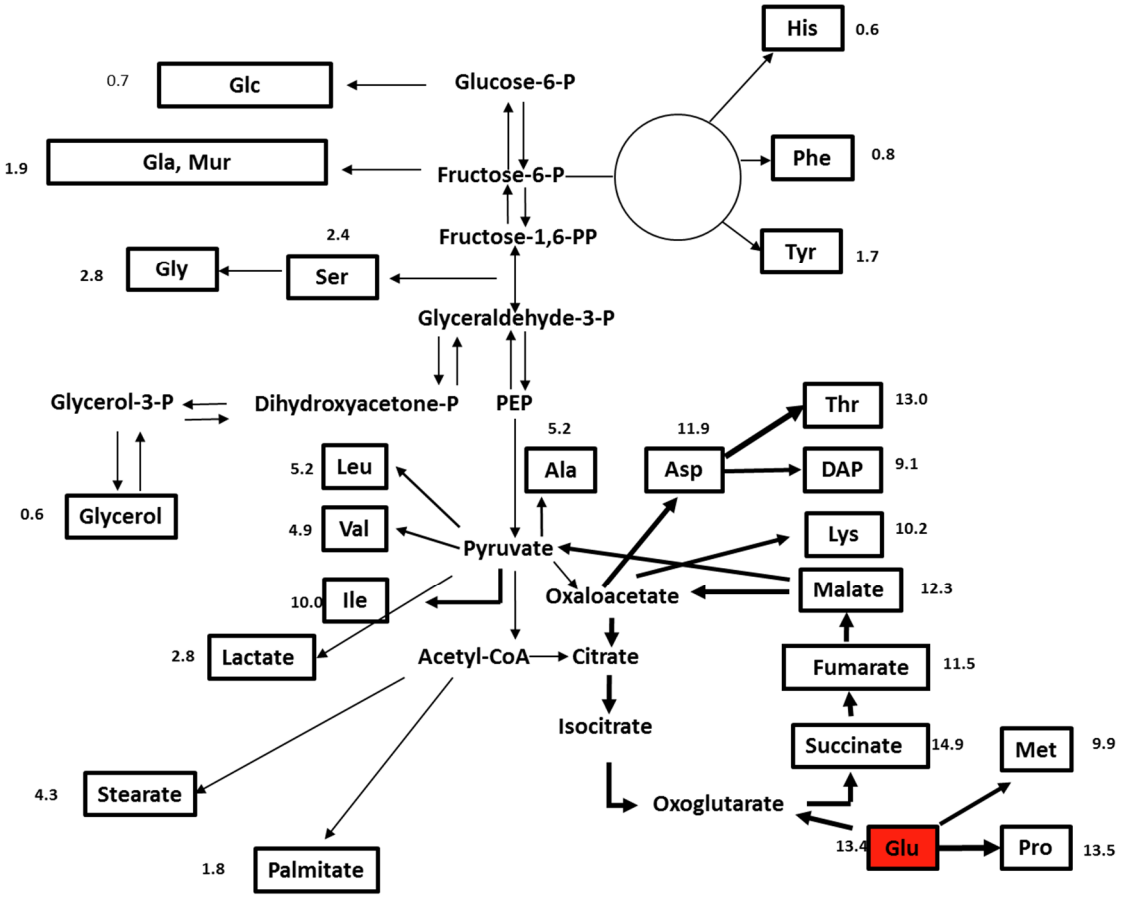
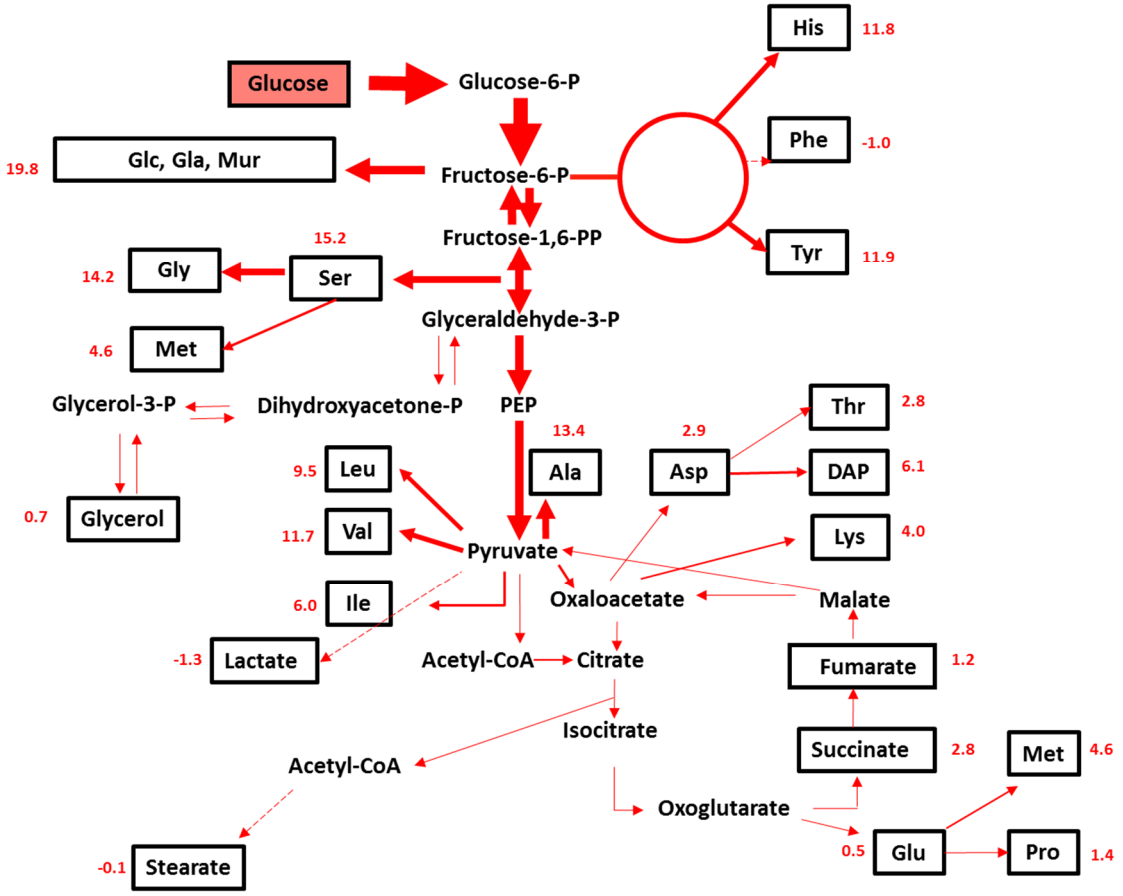
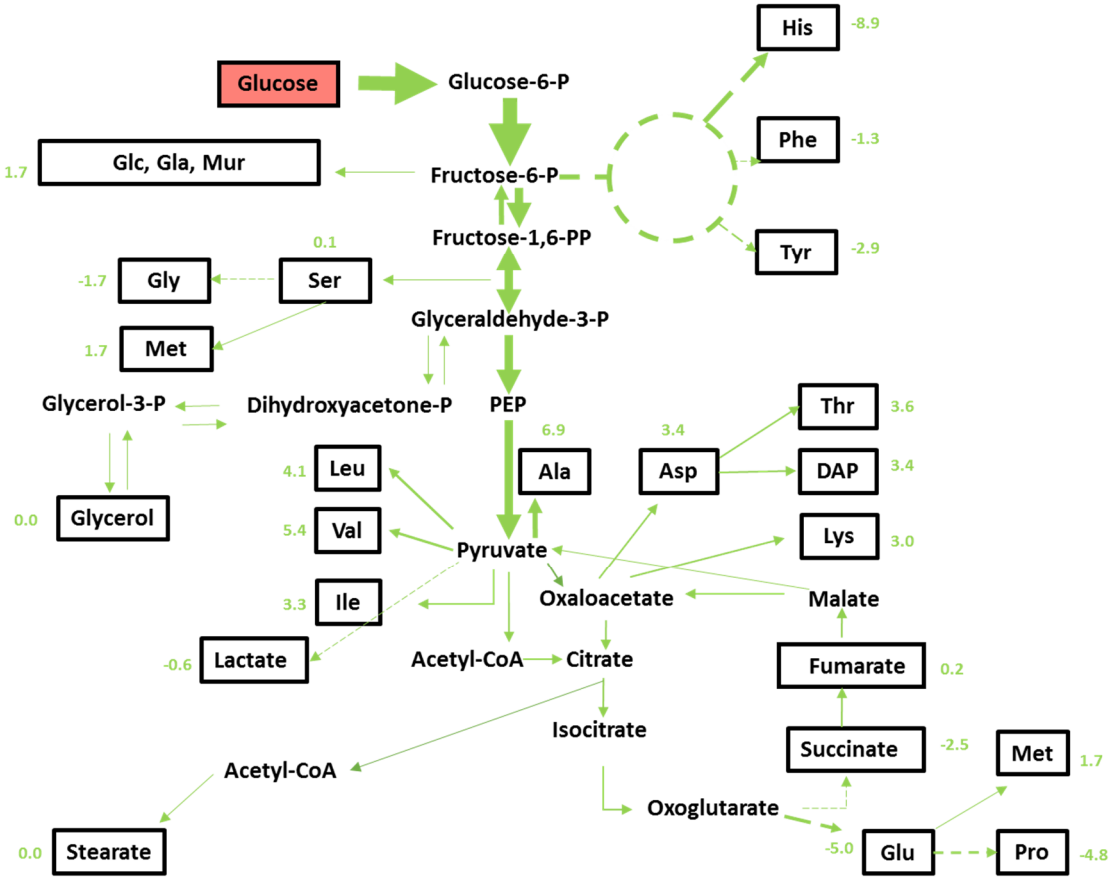


Figure 28. Observed metabolic pathways and fluxes in *B. subtilis* NCIB3610 mutant in the labelling experiments on agar containing supplied with [U-$^{13}C_6$]glucose (A) and [U-$^{13}C_5$]glutamate (B). The numbers indicate overall ^{13}C-enrichments of metabolites and the arrow widths roughly indicate the relative fluxes.

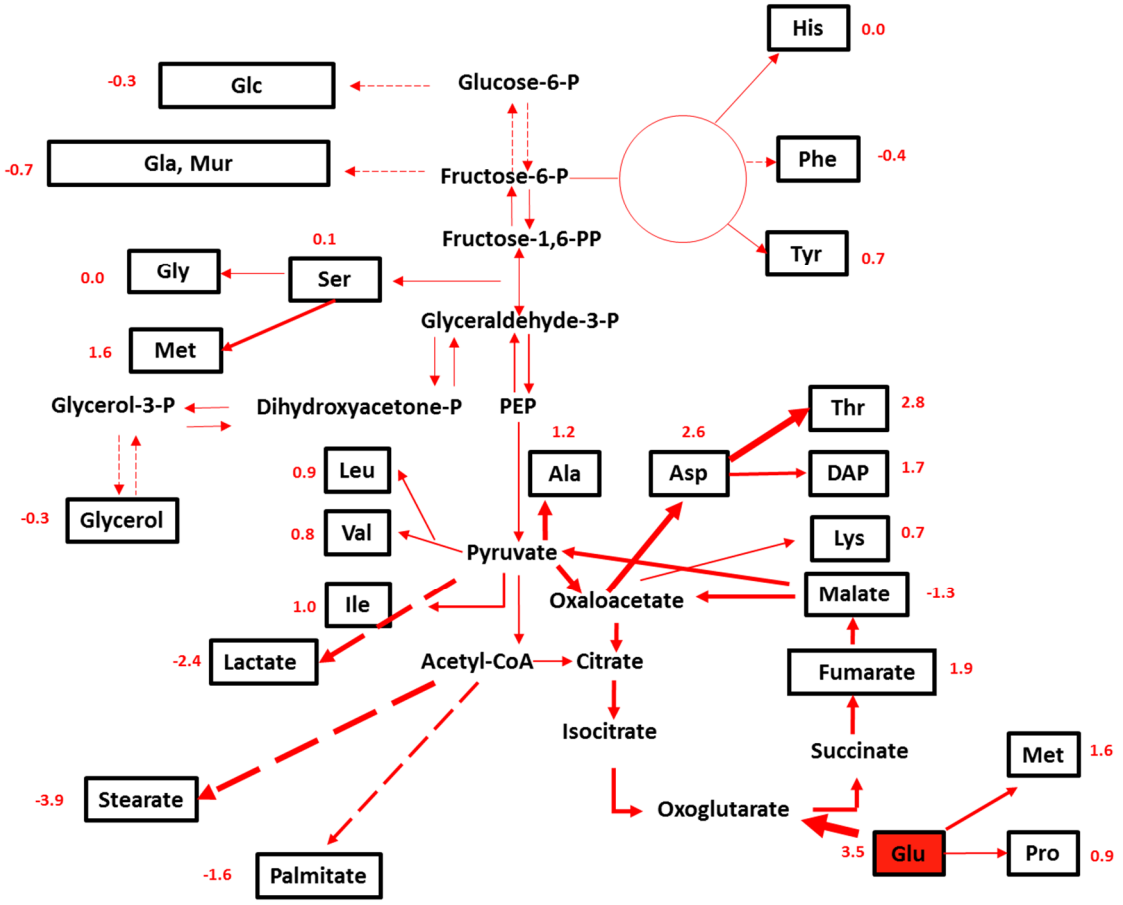
A


His
11.8
Glucose
Glucose-6-P
Phe
-1.0
19.8
Glc, Gla, Mur
Fructose-6-P
Fructose-1,6-PP
Tyr
11.9
15.2
14.2
Gly
Ser
Glyceraldehyde-3-P
4.6
Met
Glycerol-3-P
Dihydroxyacetone-P
PEP
Thr
2.8
13.4
2.9
9.5
Leu
Ala
Asp
DAP
6.1
0.7
Glycerol
11.7
Val
Pyruvate
Lys
4.0
6.0
Ile
Oxaloacetate
Malate
-1.3
Lactate
Acetyl-CoA
Citrate
Fumarate
1.2
Isocitrate
Succinate
2.8
Met
4.6
Acetyl-CoA
Oxoglutarate
0.5
Glu
Pro
1.4
-0.1
Stearate

B


Glucose
Glucose-6-P
His
-8.9
Phe
-1.3
Tyr
-2.9
1.7
Glc, Gla, Mur
Fructose-6-P
Fructose-1,6-PP
0.1
-1.7
Gly
Ser
1.7
Met
Glyceraldehyde-3-P
Glycerol-3-P
Dihydroxyacetone-P
PEP
6.9
3.4
Thr
3.6
Asp
DAP
3.4
4.1
Leu
Ala
0.0
Glycerol
5.4
Val
Pyruvate
Lys
3.0
3.3
Ile
Oxaloacetate
Malate
-0.6
Lactate
Acetyl-CoA
Citrate
Fumarate
0.2
Isocitrate
Succinate
-2.5
Met
1.7
Acetyl-CoA
Oxoglutarate
0.0
Stearate
-5.0
Glu
Pro
-4.8

C


His
0.0
-0.3
Glc
Glucose-6-P
-0.7
Gla, Mur
Fructose-6-P
Phe
-0.4
Fructose-1,6-PP
Tyr
0.7
0.1
0.0
Gly
Ser
Glyceraldehyde-3-P
1.6
Met
Glycerol-3-P
Dihydroxyacetone-P
PEP
Thr
2.8
1.2
2.6
0.9
Leu
Ala
Asp
DAP
1.7
-0.3
Glycerol
0.8
Val
Pyruvate
Lys
0.7
Malate
-1.3
1.0
Ile
Oxaloacetate
-2.4
Lactate
Acetyl-CoA
Citrate
Fumarate
1.9
Isocitrate
Succinate
Met
1.6
-3.9
Stearate
Oxoglutarate
3.5
Glu
Pro
0.9
-1.6
Palmitate

D

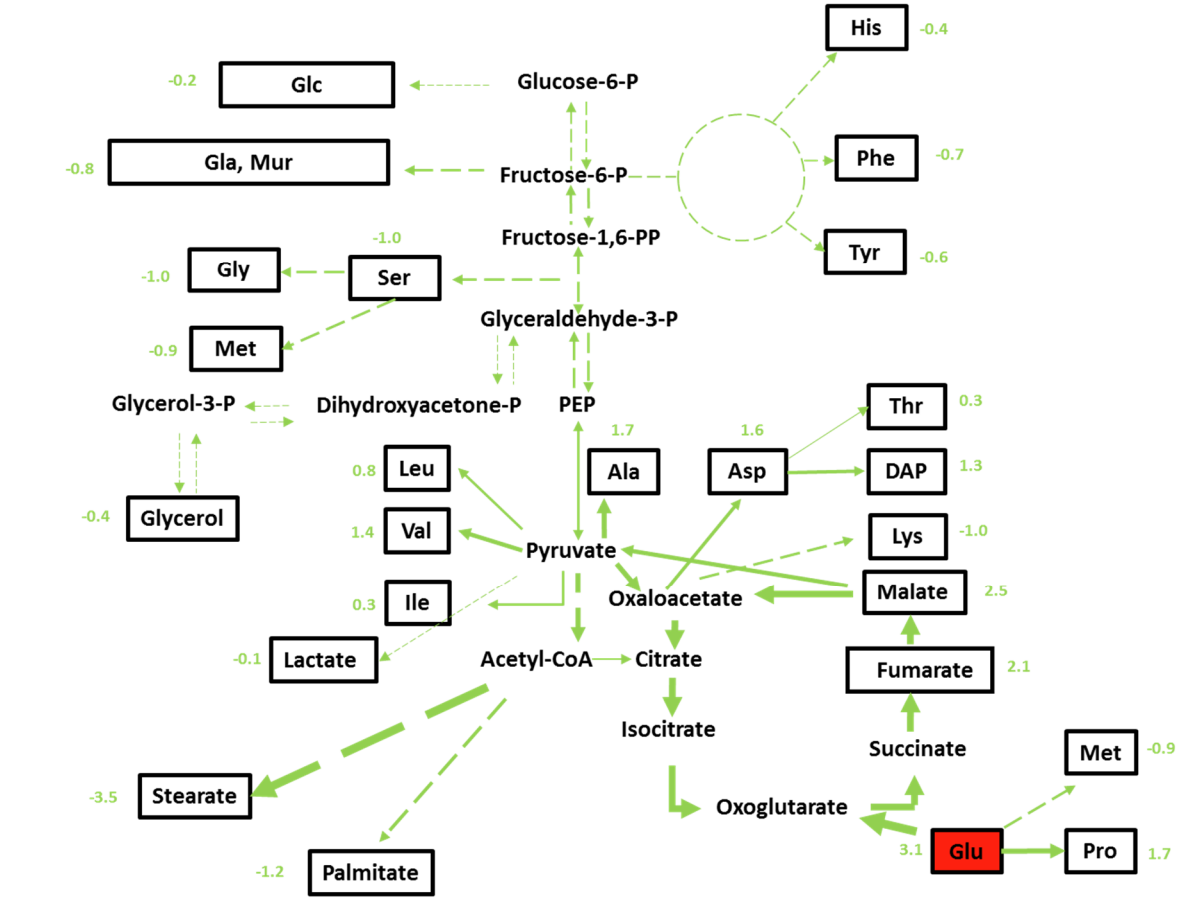


Figure 29. Observed difference of metabolic pathways and fluxes in *B. subtilis* labelling experiments. Difference in the metabolic fluxes between Δ*sinR* mutant GP1562 and NCIB3610 in the labelling experiments on agar containing supplied with [U-$^{13}C_6$]glucose (A, GP1562-NCIB3610, red arrows), and difference in the metabolic fluxes between Δ*ymdB* mutant on agar containing supplied with [U-$^{13}C_6$]glucose GP921 and NCIB3610 (B, GP921-NCIB3610, green arrows), and Difference in the metabolic fluxes between Δ*sinR* mutant GP1562 and NCIB3610 in the labelling experiments on agar containing supplied with [U-$^{13}C_5$]glutamate (C, GP1562-NCIB3610, red arrows), and difference in the metabolic fluxes between Δ*ymdB* mutant on agar containing supplied with [U-$^{13}C_5$]glutamate GP921 and NCIB3610 (D, GP921-NCIB3610, green arrows). The numbers indicate the differences in the overall ^{13}C-enrichments. The arrow widths roughly indicate the differences. Higher fluxes in GP1562/GP921 are indicated by normal arrows, lower fluxes by dashed arrows.

For the planktonic experiments, without a surface for the biofilm formation, it was shown that Δ*sinR* and Δ*ymdB* didn't play significant roles. However, on the agar plate, huge metabolic differences between the three strains were found, which could relate to the biofilm (extracellular polysaccharide with amino acids chain) formation.

3.4 Analysis of glucose uptake in immortal brown adipose tissue during isoproterenol activation using stable isotope ^{13}C as tracer

3.4.1 Experiment approach

To analyse the carbon metabolism of IBAT cells during isoproterenol activation, we performed labelling experiments with IBAT cells growing in DMEM medium containing 5 mM [U-$^{13}C_6$]glucose. More specifically, the IBAT cells were cultivated in DMEM medium for 30 min. Then, the cells were transferred into fresh DMEM medium either containing 5 mM [U-$^{13}C_6$]glucose only (control sample), or containing 5 mM [U-$^{13}C_6$]glucose and 0.5 μM isoproterenol. Each of these samples was cultivated for another 30 min at 37 °C. Finally, the medium was removed, the cells were rapidly washed with PBS solution, and the samples were quenched with the cold methanol at -78 °C. Before GC/MS measurement, the cells were dried under nitrogen gas and lyophilized. From the dried cell pellets, polar metabolites were extracted (for details, see Methods). The residue was hydrolysed under acidic conditions to obtain amino acids from the protein fraction and glucose from polysaccharide fraction. Finally, fatty acids were extracted after hydrolysis using methanolic hydrochloric acid. All of the isolated metabolites were then converted into volatile derivatives which could be analysed by GC/MS spectrometry. In addition, metabolites which were released from cells into the DMEM medium were also analysed following similar protocols as for intracellular metabolites (For details, see **Materials and Methods**).

The labelling experiments were done twice as biological replicates, each of the metabolite was measured three times by GC/MS as technical replicates. The absolute ^{13}C-enrichments significantly differed between the biological replicates, but the order of ^{13}C-excess in the detected metabolites was highly similar (see **Supplemental Tables S75–S88**). Due to the large differences in the absolute ^{13}C-enrichments, we did not calculate average values for these data. Rather, using data of the first labelling experiment, the figures were drawn.

3.4.2 ^{13}C-Profiles and quantitative analysis of the sugars

3.4.2.1 ^{13}C-Profiles of free glucose in DMEM medium

To analyse free glucose in the DMEM medium after a given labelling experiment, the filtered medium was dried under reduced pressure and the residue was treated with acetone containing sulphuric acid and acetic anhydride to convert glucose into its acetyl ester. GC-MS analysis showed that [U-$^{13}C_6$]glucose was still present in each of the experiments after the cultivation period. The measured

^{13}C-enrichment was about 15% (**Figure 30A**) with a relative fraction of >95% for the M+6 isotopologue (i.e. the molecule carrying six ^{13}C-atoms) (**Figure 30B**) for each glucose sample indicating the presence of the tracer during the whole labelling period, and, thus, establishing quasi steady-state conditions during the experiment. However, as shown in **Figure 30B** minor fractions (< 5%) of M+5 isotopologs reflected the release of small amounts of metabolized glucose from the cells into the medium. Treatment with isoproterenol had no significant impacts onto this release process, as reflected by the apparently identical ^{13}C-profiles of glucose form the medium.

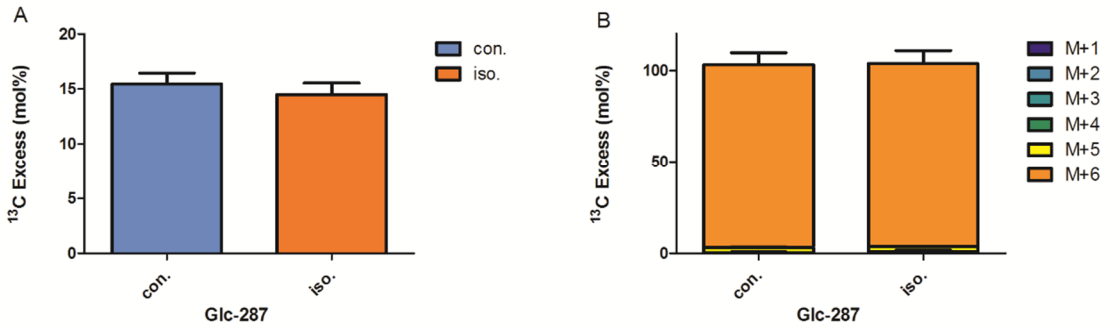


Figure 30. (A) ^{13}C-Excess (mol%) and (B) the fractional isotopolog distributions (%) of free glucose in the used DMEM medium supplemented with 5 mM [U-$^{13}C_6$]glucose. ^{13}C-Excess (mol%) for the control sample (without isoproterenol) or the sample from the experiment with isoproterenol. ^{13}C-Excess (mol%) and relative fractions of isotopologs (%) were determined by GC/MS of the glucose derivative at the indexed m/z values. Error bars indicate standard deviations from the means of 3 values (3 × technical replicates). M+1, M+2, M+3, etc. indicate isotopologs carrying 1, 2, 3, etc. ^{13}C-atoms, respectively. For numerical values, see **Supplemental Tables S85**, **S86**.

3.4.2.2 ^{13}C-Profiles of glycogen derived glucose in the IBAT cell

After mild acidic hydrolysis, glycogen-derived glucose was obtained from the dried cell pellet and again converted into its acetate ester by the protocol described in Methods. In comparison to the free glucose from the DMEM medium, glycogen derived glucose from the cells was lower labelled (in the control sample (con.) about 1%, in the isoproterenol sample (iso.) about 3%) (**Figure 31A**, and **Supplemental Tables S83**). In both samples, the major isotopolog was again M+6 (**Figure 31B**) reflecting direct incorporation of [U-$^{13}C_6$]glucose into glycogen via [U-$^{13}C_6$]glucose 6-phosphate. Notably, however, significant differences between the relative fractions of M+1 to M+5 isotopologs could be noticed (**Figure 31B**). More specifically, these isotopologs accounted in total for about 20% in the con. sample, whereas in the iso. sample this fraction was less than 10%. On this basis, it can be concluded that isoproterenol resulted in higher rates of the direct usage of [U-$^{13}C_6$]glucose for glycogen synthesis with lower rates of glycolytic cycling prior to polymer formation.

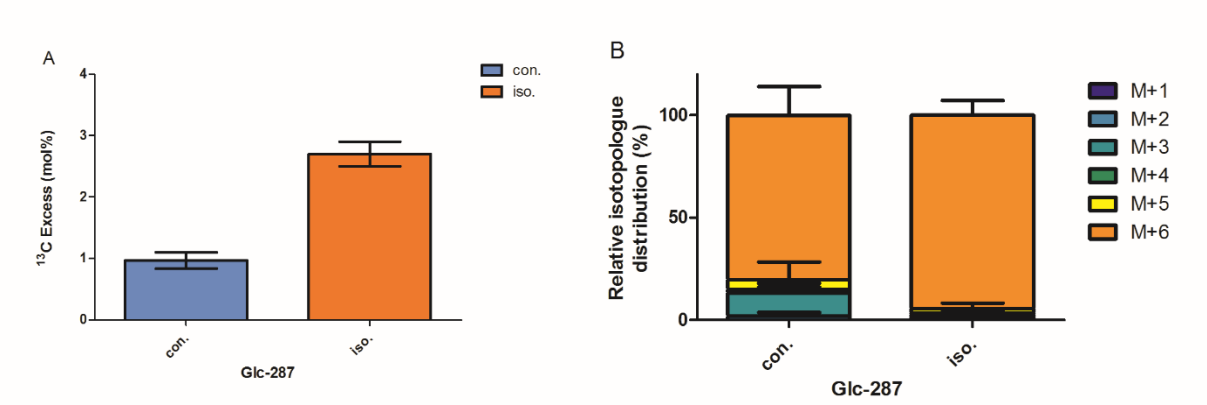


Figure 31. (A) ^{13}C-Excess (mol%) and (B) the fractional isotopolog distributions (%) of glycogen derived glucose in the labelling experiment supplement with 5 mM [U-$^{13}C_6$]glucose. ^{13}C-Excess (mol%) for the control sample (without isoproterenol) or the sample from the experiment with isoproterenol. ^{13}C-Excess (mol%) and relative fractions of isotopologs (%) were determined by GC/MS of the glucose derivative at the indexed m/z values. Error bars indicate standard deviations from the means of 3 values (3 × technical replicates). M+1, M+2, M+3, etc. indicate isotopologs carrying 1, 2, 3, etc. ^{13}C-atoms, respectively. For numerical values, see **Supplemental Tables S83**, **S84**.

3.4.3 Isotopolog profiles of polar metabolites

3.4.3.1 Quantitative analysis of polar metabolites in DMEM medium and IBAT cells

Using a mixture of methanol, water and chloroform, more than 20 polar metabolites were extracted from the dried IBAT cell pellets or the DMEM medium, including free amino acids, lactate, glycerol, TCA intermediates and free fatty acids. To quantify the amounts of polar metabolites in the cell extracts and the used DMEM medium, norvaline was added as an internal standard (see also **Supplemental Tables S81**, **S82**). Figure 32 shows the amount of detectable polar metabolites (µmol/well) isolated from DMEM medium (**Figure 32A**) or from the IBAT cell (**Figure 32B**).

In each of the samples, the amounts of lactate, valine, leucine, isoleucine and free fatty acids were significant higher than those of the other detected polar metabolites (**Figure 32A**). Interestingly, in the medium collected from the experiments with isoproterenpol the amounts of most polar metabolites (e.g. lactate, valine, leucine, isoleucine and free fatty acids) were much higher (by an approximate factor of 6) than in the medium from the control sample (**Figure 32A**, **Supplemental Tables S82**).

Similarly, the amounts of lactate and free fatty acids were also much higher in extracts from IBAT cells cultivated in the presence of isoproterenol. In sharp contrast, the amounts of free amino acids

detected in the cell extracts were almost the same in cells from the experiments with or without isoproterenol. It follows that isoproterenol treatment lead to the formation and release of more lactate and free fatty acids.

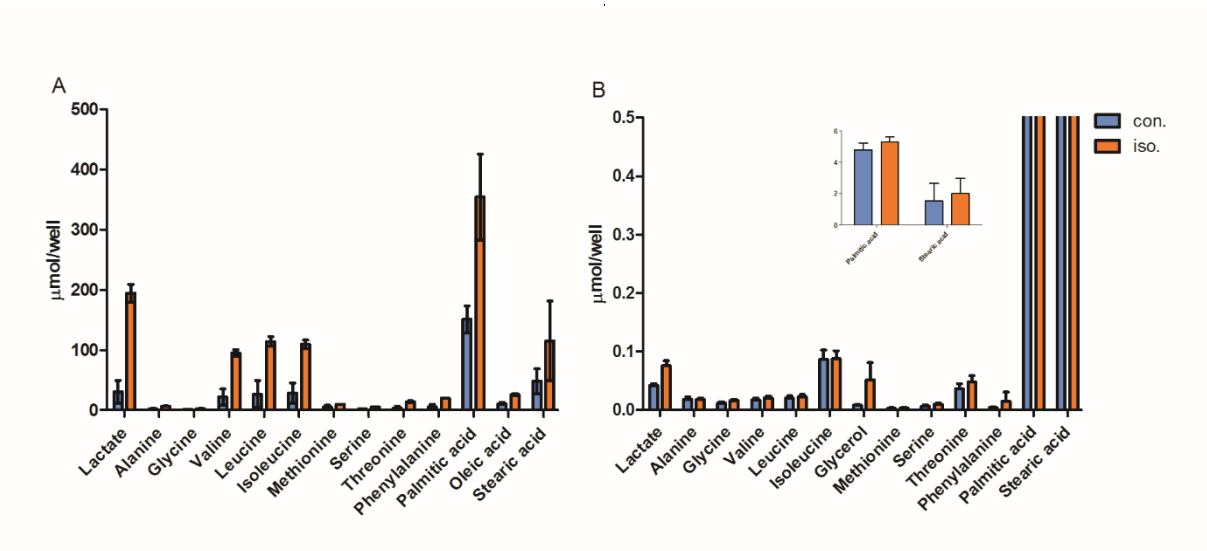


Figure 32. (A) The amount (umol) of polar metabolites in con. and iso. immortal brown adipose tissue per well grown in DMEM medium supplied with 10 mM [U-$^{13}C_6$]glucose. (B) The amount (umol) was determined by GC/MS using silylated internal standard norvaline. Error bars indicate standard deviations from the means of 3 values 3 × technical replicates. For numerical values, see **Supplemental Tables S82**, **S83**.

3.4.3.2 ^{13}C-Enrichment of free polar metabolites in DMEM medium and IBAT cell samples

After the quantitative analysis of the free metabolites, the samples were measured again three times using a sim-method (details, see under Methods) to obtain the values for ^{13}C-excess and isotopolog distributions. **Figure 33A** shows the ^{13}C-excess values for polar metabolites from the used DMEM medium. Because of the high amounts of unlabelled amino acids in the DMEM medium (**Supplemental Table 82**), the ^{13}C-excess of most amino acids were very low, except for Ala and Phe (about 1%). However, lactate and glycolate from both DMEM samples in the control and the isoproterenol treated experiments displayed significant ^{13}C-incorporation (ca. 11 %) (**Supplemental Tables S79**) reflecting their biosynthetic origin from the glucose tracer and their subsequent release into the medium. Labelled lactate was observed as M+3 isotopolog which can be explained by degradation of [U-$^{13}C_6$]glucose into [U-$^{13}C_3$]pyruvate followed by reduction into [U-$^{13}C_3$]lactate. Glycolic acid was mainly M+2 labelled, which indicates its origin from [U-$^{13}C_2$]acetyl-CoA. Ala was also formed from pyruvate as reflected by the similar isotopolog distributions in alanine and lactate. The isotopolog composition of Phe was more complex with a main fraction of M+3, reflecting the usage of [U-$^{13}C_3$]PEP as a biosynthetic precursor.

Figure 33B shows the ^{13}C-excess of polar metabolites isolated from IBAT cells. Similar to the findings of metabolites from the medium, most amino acids from the cells were also less labelled, except for Ala, Asp, Glu and Phe with ^{13}C-excess of 2-5% (**Supplemental Tables S77**). Slightly higher ^{13}C-excess values were observed for metabolites from the con. sample.

Same as for Ala from the DMEM medium, the major isotopolog in Ala isolated from IBAT cells was M+3 confirming the glycolytic pathway from [U-$^{13}C_6$]glucose. Asp and Glu were mainly found as M+1 and M+2 species, with minor amounts of M+3 suggesting the formation via $^{13}C_3$-oxaloacetate or [U-$^{13}C_2$]acetyl-CoA after a round of the TCA cycle. As well as Phe from the medium, Phe from the IBAT cells was detected as M+3 and M+5 isotopologues. However, Phe from the iso. cells had significant higher ^{13}C-enrichment (4.9 %). Lactate showed very high ^{13}C-excess (11 %) from both con. and iso. IBAT cells and displayed high fractions of M+3 species via their formation from [U-$^{13}C_3$]pyruvate. ^{13}C-Flux from glucose into the labelled TCA intermediates, succinate, fumarate and malate could also be detected, albeit the differences of ^{13}C-excess between the con. and iso. samples were not high. Free fatty acids were only less labelled (< 0.5%), whereas 3-hydroxybutyrate, also predicted to be formed from acetyl-CoA, was highly labelled (from con. sample about 7%, from iso. sample 4%). The main fraction of M+2 isotopologs in 3-hydroxybutyrate confirmed its origin from [U-$^{13}C_2$]acetyl-CoA.

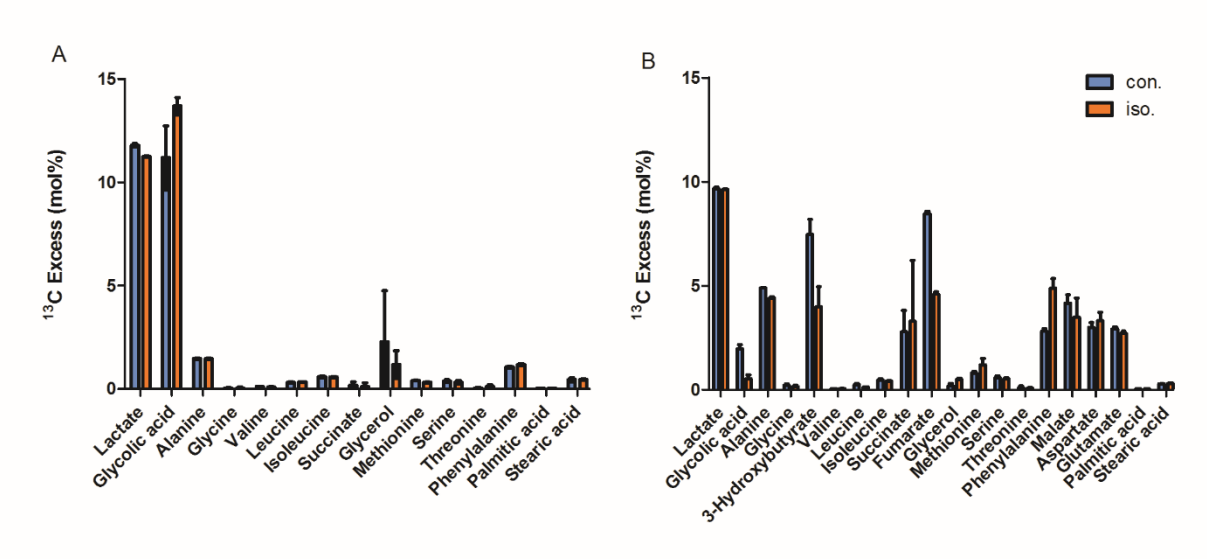


Figure 33. (A) ^{13}C-Excess (mol%) in polar metabolites in used DMEM medium (B) ^{13}C-Excess (mol%) in polar metabolites of IBAT cell grown in DMEM medium supplemented with 5 mM [U-$^{13}C_6$]glucose. ^{13}C-Excess (mol%) for the control sample (without isoproterenol) or the sample from the experiment with isoproterenol. ^{13}C-Excess (mol%) and relative fractions of isotopologs (%) were determined by GC/MS of the glucose derivative at the indexed m/z values. Error bars indicate standard deviations from the means of 3 values (3 × technical replicates). M+1, M+2, M+3, etc. indicate isotopologs carrying 1, 2, 3,

etc. ^{13}C-atoms, respectively. For numerical values, see **Supplemental Tables S77**, **S79**.

To estimate relative flux contributions from pyruvate to oxaloacetate or acetyl-CoA, respectively, we calculated the ratio of M+2 and M+3 fractions in aspartate and malate. Since oxaloacetate could not be detected due to the low steady-state concentration, we analysed Asp and malate instead of oxaloacetate. Asp can be formed from pyruvate via oxaloacetate which contained M+1, M+2 and M+3 isotopolog fractions. Thus, the M+3 fraction in Asp reflects the M+3 fraction in oxaloacetate using [U-$^{13}C_3$]pyruvate as a precursor. On the other hand, the M+2 fraction in Asp reflects the M+2 fraction in oxaloacetate made from [U-$^{13}C_2$]acetyl-CoA via the TCA cycle. Since malate is directly related to oxaloacetate by malate dehydrogenase, the M+3 fraction in malate also represents the M+3 fraction in oxaloacetate. Similarly, the M+2 fraction in malate represents the M+2 fractions in acetyl-CoA. Therefore, the ratios of M+3 and M+2 fractions in Asp and malate, respectively, illustrate the relative fluxes from pyruvate to oxaloacetate or acetyl-CoA, respectively.

$$ratio\ of\ M+3\ to\ M+2 = \frac{(M+3)}{(M+2)}$$

Table 3. Ratio of M+3 fraction to M+2 fraction in Asp and malate

Ratio	Con.	Iso.
Asp	0.76	0.36
Malate	1.69	0.63

The ratio of M+3 to M+2 in Asp and malate were both higher in the control samples from the experiments without isoproterenol. This indicates that isoproterenol redirected the carbon flux to malate/oxaloacetate from acetyl-CoA via the TCA cycle.

3.4.3 Isotopolog profiles of protein derived amino acids in the IBAT cell

Using hydrochloric acid, the con. and iso. IBAT cell samples were hydrolysed, resulting in a mixture of amino acids. Following our standard protocols, amino acids were converted into TBDMS-derivatives which were then analysed by GC/MS. During the hydrolysis, Cys and Trp were destroyed and could not be detected. Because of the deamidation, Asn and Gln were converted into Asp and Glu. Because of the big amount of different amino acids presented in the DMEM medium (**Supplemental Table 89**) and the short time (i.e. 30 min) for the labelling experiment, all protein derived amino acids from con. and iso. samples were very low labelled (less than 0.8%)

(**Supplemental Tables S75**). Surprisingly, all the protein derived amino acids had similar isotopolog distributions, which were characterized by high amounts (more than 90%) of M+1 isotopologs (**Supplemental Tables S76**). This could point at contributions of $^{13}CO_2$ being incorporated into protein-bound amino acids by reversible reactions of amino acids degradation pathways.

3.4.4 Isotopolog profiles of lipid derived fatty acids in the IBAT cell

A mixture of methyl ester derivatives of lipid derived fatty acids could be obtained from the IBAT cell samples treated with methanolic hydrochloric acid. Because of the short time for the labelling experiment (i.e. 30 min) and the rich supply of unlabelled substrates in the DMEM medium, all lipid derived fatty acids from con. and iso. samples were very low labelled (less than 0.5%) (**Supplemental Tables S87**). Notably, the isotopolog distributions of fatty acids mostly displayed M+1 labelling (more than 90%) (**Supplemental Tables S88**), which was reminiscent of the patterns found for protein derived amino acids. Again, this might also indicate $^{13}CO_2$ incorporation.

In total, from the labelling patterns of different metabolites from [U-$^{13}C_6$]glucose, glucose is shown as an efficient precursor for IBAT cells to be metabolized into glycogen (especially under treatment with isoproterenol). Moreover, the ^{13}C-profiles reflected carbon flux from exogenous glucose via the glycolytic pathway to form pyruvate/Ala/lactate and acetyl-CoA, serving as a precursor for fatty acids and TCA intermediates.

3.4.5 Discussion

During the whole period of the labelling experiments, the glucose tracer was present in high amount, thus establishing a constant source for carbon metabolism under quasi steady state conditions. The labelling profiles of glycogen-derived glucose suggest that isoproterenol stimulated glycogen biosynthesis directly using glucose from the medium without prior metabolization and cycling. On this basis, it can be speculated that the cells prefer to store the nutrient glucose as glycogen and use fatty acids as the energy source during isoproterenol treatment.

The data also suggest that isoproterenol treatment stimulates lactate formation from exogenous glucose and its subsequent release from the cells. During the fermentation of the IBAT cells, lactate could be produced and isoproterenol probably promotes the rate of fermentation. Moreover, in most animals lactate can be release into the blood stream, serving as energy source by reconverting it into glucose (Andersen et al 2013). Notably, our findings concerning lactate production is also in line to earlier observations (Larsen, 1999).

Because of the big amount of most amino acids in the medium (see **Supplemental Table S89**), released amino acids were diluted and could not significantly contribute to the fraction of ^{13}C-labelled amino acids in the medium. However, Ala which is not present in the DMEM could be found in the medium in labelled form (about 1 % ^{13}C excess). Interestingly, also Phe from the DMEM medium acquired some ^{13}C-label (about 1 % ^{13}C excess). It can be speculated that IBAT cells generally released amino acids at minor rates to the medium. However, the ^{13}C-enrichments were unchanged in the experiments with or without isoproterenol.

On the basis of the labelling profiles in free aspartate and malate, it can be concluded that pyruvate enters the TCA cycle via two pathways. In the con. cells, a major fraction of aspartate and malate is derived via oxaloacetate formed by carboxylation of pyruvate leading to M+3 isotopologs. In contrast, in the iso. sample aspartate and malate show larger fractions of the M+2 species which can be explained by carbon flux of pyruvate to acetyl-CoA entering the TCA cycle. It can be speculated that the isoproterenol treatment increased the carbon flux via the TCA for higher demands of energy. Consequently, more CO_2 is formed and released in the isoproterenol samples. Protein derived amino acids and lipid derived fatty acids from both con. and iso. samples were very low labelled (< 0.8 %, **Supplemental Table S75**, **S87**). Interestingly, these low amounts of labelled compounds carried only one ^{13}C-atom, i.e. were present as M+1 forms. On this basis, it can be concluded that in both samples, $^{13}CO_2$ formed via the TCA cycle from [U-$^{13}C_6$]glucose could finally enter protein derived amino acids and lipid derived fatty acids via anaplerotic reactions using $^{13}CO_2$ as a precursor. This might reflect an important role of CO_2 in the metabolism of IBAT.

In conclusion, the labelling experiments showed some significant impacts of isoproterenol on the core metabolic fluxes of IBAT cells. The key findings are: (i) glucose flux into glycogen storage is increased, (ii) more lactate is produced from glucose, and (iii) carbon flux is directed into the oxidative TCA cycle probably to generate more energy.

List of abbreviations

°C	degrees Celsius
%	percent
µL	microliter
µm	micrometer
3-HBA	3-hydroxybutyrate
6-PG	6-phosphogluconate
ADP	adenosine diphosphate
Ala	alanine
Arg	arginine
Asn	asparagine
Asp	aspartate
ATP	adenosine triphosphate
Cys	cysteine
DAP	diaminopimelic acid
DNA	deoxyribonucleic acid
DHAP	dihydroxyacetone phosphate
ED pathway	Entner-Doudoroff pathway
$FADH_2$	flavin adenine dinucleotide
Fru-6-P	fructose 6-phosphate
g	gram

G3P	glycerol 3-phosphate
GAP	glyceraldehyde 3-phosphate
GC/MS	gas chromatography/mass spectrometry
GDP	guanosine diphosphate
GlcN	glucosamine
Gln	glutamine
glpD	glycerol 3-phosphate dehydrogenase
glpK	glycerol kinase
Glu	glutamate
Glu-6-P	glucose 6-phosphate
Gly	glycine
h	hour
His	histidine
Ile	isoleucine
KDPG	2-keto-3-deoxy-6-phosphogluconate
L	liter
Leu	leucine
Lys	lysine
M	molar
Met	methionine
mg	milligram
min	minutes

mL	milliliter
mm	millimeter
mM	millimolar
mRNA	messenger RNA
Mur	muramic acid
NAD(P)H	nicotinamide adenine dinucleotide (phosphate)
ng	nanogram
OD600	optical density at 600 nanometer
PBS	phosphate-buffered saline
PEP	phosphoenolpyruvate
pH	Potential of hydrogen
PHB	polyhydroxybutyrate
Phe	phenylalanine
PPP	pentose phosphate pathway
Pro	proline
RNA	ribonucleic acid
rpe	ribulose-phosphate 3-epimerase
rpiA	ribulose 5-phophate isomerase
rpm	rounds per minute
RT	retention time
s	seconds
SD	standard deviation

Ser	serine
TBDMS	tert-butyl-dimethylsilyl
TCA	tricarboxylic acid
Thr	threonine
TMS	trimethylsilyl
tRNA	transfer RNA
Trp	tryptophane
Tyr	tyrosine
Val	valine
α-KGA	α-ketoglutarate

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Supporting Information

Supplemental Tables S1 ^{13}C-Excess (mol%) of protein-bound amino acids from [U-$^{13}C_6$]glucose

^{13}C-Excess (mol%) of protein-bound amino acids from experiments with *Fth* WT, *Fth* LVS and *Fno* grown in medium T supplemented with 11 mM [U-$^{13}C_6$]glucose. Mean and SD from three independent experiments are shown.

11 mM [U-$^{13}C_6$]glucose	*Fth* WT	*Fth* LVS	*Fno*
Ala	7.82% ± 0.37%	7.87% ± 0.59%	9.05% ± 0.79%
Asp	1.68% ± 0.33%	1.50% ± 0.64%	0.72% ± 0.32%
Glu	3.91% ± 0.24%	3.82% ± 0.22%	4.15% ± 0.65%
Gly	0.35% ± 0.13%	0.14% ± 0.12%	0.83% ± 0.19%
His	0.14% ± 0.06%	0.10% ± 0.12%	0.06% ± 0.03%
Ile	0.09% ± 0.02%	0.08% ± 0.05%	0.08% ± 0.05%
Leu	0.00% ± 0.00%	0.01% ± 0.01%	0.01% ± 0.01%
Lys	0.05% ± 0.01%	0.09% ± 0.02%	0.07% ± 0.03%
Phe	0.30% ± 0.02%	0.13% ± 0.22%	0.06% ± 0.12%
Pro	0.15% ± 0.01%	0.30% ± 0.11%	0.14% ± 0.04%
Met		0.49% ± 0.26%	0.50% ± 0.31%
Ser	0.17% ± 0.03%	0.13% ± 0.05%	0.87% ± 0.24%
Thr	0.63% ± 0.02%	0.58% ± 0.06%	0.66% ± 0.12%
Tyr	1.13% ± 0.07%	0.77% ± 0.07%	2.48% ± 0.29%
Val	0.21% ± 0.01%	0.05% ± 0.07%	0.12% ± 0.10%

Supplemental Tables S2 ^{13}C-Excess (mol%) of protein-bound amino acids from [1,2-$^{13}C_2$]glucose

^{13}C-Excess (mol%) of protein-bound amino acids from experiments with *Fth* WT, *Fth* LVS and *Fno* grown in medium T supplemented with 11 mM [1,2-$^{13}C_2$]glucose. Mean and SD from two independent experiments are shown.

11 mM [1,2-$^{13}C_2$]glucose	*Fth* WT	*Fth* LVS	*Fno*
Ala	2.62% ± 0.09%	3.44% ± 0.12%	3.63% ± 0.07%
Asp	0.57% ± 0.00%	1.02% ± 0.27%	0.47% ± 0.09%
Glu	1.62% ± 0.10%	2.37% ± 0.14%	2.54% ± 0.03%
Gly	0.31% ± 0.03%	0.02% ± 0.01%	0.20% ± 0.02%

His	0.14% ± 0.08%	0.15% ± 0.00%	
Ile	0.12% ± 0.00%	0.04% ± 0.01%	0.12% ± 0.00%
Leu	0.00% ± 0.00%	0.03% ± 0.00%	0.07% ± 0.00%
Lys	0.08% ± 0.01%	0.20% ± 0.01%	
Phe	0.23% ± 0.00%	0.16% ± 0.04%	0.34% ± 0.05%
Pro	1.25% ± 0.46%	0.20% ± 0.07%	0.81% ± 0.11%
Met	0.18% ± 0.01%		
Ser	0.20% ± 0.01%	0.38% ± 0.01%	1.28% ± 0.07%
Thr	0.72% ± 0.02%	0.13% ± 0.03%	0.44% ± 0.13%
Tyr	0.53% ± 0.09%	0.65% ± 0.43%	1.72% ± 0.05%
Val	0.17% ± 0.04%	0.02% ± 0.01%	0.09% ± 0.01%

Supplemental Tables S3 ^{13}C-Excess (mol%) of protein-bound amino acids from [U-$^{13}C_3$]serine

^{13}C-Excess (mol%) of protein-bound amino acids from experiments with *Fth* WT, *Fth* LVS and *Fno* grown in medium T supplemented with 3 mM [U-$^{13}C_3$]serine. Mean and SD from three independent experiments are shown.

3 mM [U-$^{13}C_3$]serine	*Fth* WT	*Fth* LVS	*Fno*
Ala	5.13% ± 0.06%	5.79% ± 0.12%	1.44% ± 0.04%
Asp	0.99% ± 0.03%	1.20% ± 0.08%	0.14% ± 0.08%
Glu	2.09% ± 0.02%	2.62% ± 0.02%	0.37% ± 0.04%
Gly	3.68% ± 0.25%	2.68% ± 0.27%	8.28% ± 0.64%
His	0.07% ± 0.01%	0.04% ± 0.01%	0.05% ± 0.00%
Ile	0.07% ± 0.00%	0.11% ± 0.03%	0.10% ± 0.01%
Leu	0.01% ± 0.01%	0.03% ± 0.03%	0.00% ± 0.00%
Lys	0.10% ± 0.02%	0.09% ± 0.01%	0.09% ± 0.01%
Phe	3.32% ± 0.52%	0.55% ± 0.09%	0.17% ± 0.06%
Pro	0.11% ± 0.03%	0.07% ± 0.00%	0.18% ± 0.01%
Met	0.21% ± 0.02%	0.20% ± 0.02%	0.25% ± 0.09%
Ser	12.92% ± 0.12%	12.39% ± 0.20%	16.02% ± 0.57%
Thr	0.53% ± 0.04%	0.58% ± 0.08%	0.51% ± 0.12%

Tyr	0.62% ± 0.55%	0.16% ± 0.09%	0.38% ± 0.28%
Val	0.05% ± 0.01%	0.06% ± 0.06%	0.06% ± 0.04%

3 mM [U-$^{13}C_3$]serine	*Fth* WT and *Fth* LVS	*Fno*
Ser	12.66% ± 0.37%	16.02% ± 0.57%

Supplemental Tables S4 ^{13}C-Excess (mol%) of protein-bound amino acids from [U-$^{13}C_3$] glycerol

^{13}C-Excess (mol%) of protein-bound amino acids from experiments with *Fth* WT, *Fth* LVS and *Fno* grown in medium T supplemented with 25 mM [U-$^{13}C_3$] glycerol. Mean and SD from three independent experiments are shown.

25 mM [U-$^{13}C_3$]glycerol	*Fth* WT	*Fth* LVS	*Fno*
Ala	4.79% ± 0.97%	3.51% ± 0.08%	22.54% ± 2.41%
Asp	1.17% ± 0.09%	0.65% ± 0.20%	1.19% ± 0.58%
Glu	2.39% ± 0.51%	1.82% ± 0.13%	9.25% ± 1.61%
Gly	0.25% ± 0.11%	0.14% ± 0.01%	2.30% ± 0.45%
His	0.08% ± 0.02%	0.08% ± 0.00%	0.13% ± 0.12%
Ile	0.09% ± 0.03%	0.07% ± 0.04%	0.11% ± 0.02%
Leu	0.05% ± 0.04%	0.06% ± 0.03%	0.03% ± 0.02%
Lys	0.09% ± 0.03%	0.14% ± 0.01%	0.09% ± 0.04%
Phe	0.62% ± 0.41%	0.34% ± 0.34%	0.18% ± 0.14%
Pro	0.17% ± 0.06%	0.33% ± 0.03%	0.20% ± 0.11%
Met	0.33% ± 0.00%	0.29% ± 0.01%	0.25% ± 0.03%
Ser	0.17% ± 0.05%	0.16% ± 0.03%	2.56% ± 0.56%
Thr	0.46% ± 0.08%	0.22% ± 0.13%	0.49% ± 0.09%
Tyr	0.55% ± 0.02%	0.41% ± 0.08%	5.12% ± 0.33%
Val	0.03% ± 0.00%	0.04% ± 0.02%	0.24% ± 0.02%

Supplemental Table S5 ^{13}C-Excess (mol%) of polar metabolites from [U-$^{13}C_6$]glucose

^{13}C-Excess (mol%) of polar metabolites from experiments with *Fth* WT, *Fth* LVS and *Fno* grown in medium T supplemented with 11 mM [U-$^{13}C_6$]glucose. Mean and SD from three independent experiments are shown.

11 mM [U-$^{13}C_6$]glucose	*Fth* WT	*Fth* LVS	*Fno*
Lactate	7.31% ± 0.78%	7.67% ± 1.39%	7.80% ± 0.70%
Glycolic acid	10.54% ± 0.46%	9.39% ± 1.30%	9.40% ± 1.65%
Alanine	9.96% ± 0.45%	9.89% ± 0.11%	10.00% ± 0.82%
Glycine	0.27% ± 0.03%	0.20% ± 0.22%	0.67% ± 0.27%
Oxalic acid	2.61% ± 0.18%	2.04% ± 0.21%	1.54% ± 0.43%
3-hydroxy butyric acid	9.71% ± 0.42%	10.14% ± 0.26%	11.94% ± 0.70%
Valine	0.18% ± 0.02%	0.06% ± 0.06%	0.07% ± 0.07%
leucine	0.03% ± 0.00%	0.02% ± 0.01%	0.01% ± 0.01%
Isoleucine	0.03% ± 0.01%	0.03% ± 0.02%	0.05% ± 0.02%
Succinic acid	3.78% ± 0.33%	5.70% ± 0.91%	6.36% ± 1.16%
Fumaric acid	1.09% ± 0.78%	1.65% ± 0.29%	2.07% ± 0.72%
Glycerol	8.15% ± 0.49%	7.64% ± 0.84%	9.29% ± 1.25%
Methionine	0.12% ± 0.03%	0.08% ± 0.06%	0.08% ± 0.03%
Serine	0.53% ± 0.04%	0.45% ± 0.04%	0.56% ± 0.06%
Threonine	0.20% ± 0.02%	0.16% ± 0.09%	0.19% ± 0.06%
Phenylalanine	1.14% ± 0.10%	2.27% ± 0.30%	0.20% ± 0.05%
Malic acid	2.81% ± 0.20%	2.95% ± 0.31%	7.65% ± 1.75%
Aspartate	0.41% ± 0.02%	0.36% ± 0.20%	0.09% ± 0.05%
Glutamate	6.21% ± 0.27%	4.61% ± 0.63%	3.48% ± 0.33%
Palmitic acid	6.50% ± 0.47%	5.43% ± 1.48%	6.25% ± 1.03%
Oleic acid	6.92% ± 0.67%	6.77% ± 0.40%	7.50% ± 0.47%
Stearic acid	8.03% ± 1.46%	7.22% ± 1.50%	8.06% ± 0.77%
Citric acid	0.16% ± 0.09%	0.35% ± 0.08%	0.68% ± 0.44%

Supplemental Table S6 ^{13}C-Excess (mol%) of polar metabolites from [1,2-$^{13}C_2$]glucose

^{13}C-Excess (mol%) of polar metabolites from experiments with *Fth* WT grown in medium T supplemented with 11 mM [1,2-$^{13}C_2$]glucose. Mean and SD from two independent experiments are shown.

11 mM [1,2-$^{13}C_2$]glucose	*Fth* WT	*Fth* LVS	*Fno*

Lactate	5.04% ± 0.38%		
Glycolic acid	4.87% ± 0.12%		
Alanine	3.16% ± 0.23%		
Glycine	0.13% ± 0.03%		
Oxalic acid	0.01% ± 0.01%		
3-hydroxy butyric acid	3.51% ± 0.12%		
Valine	0.16% ± 0.00%		
leucine	0.03% ± 0.00%		
Isoleucine	0.04% ± 0.00%		
Succinic acid	0.53% ± 0.04%		
Fumaric acid	0.24% ± 0.01%		
Glycerol	2.74% ± 0.23%		
Methionine	0.11% ± 0.01%		
Serine	0.43% ± 0.04%		
Threonine	0.18% ± 0.01%		
Phenylalanine	0.48% ± 0.04%		
Malic acid	1.24% ± 0.02%		
Aspartate	0.19% ± 0.02%		
Glutamate	2.04% ± 0.17%		
Palmitic acid	2.90% ± 0.17%		
Oleic acid	3.44% ± 0.26%		
Stearic acid	3.98% ± 0.21%		
Citric acid			

Supplemental Table S7 ^{13}C-Excess (mol%) of polar metabolites from [U-$^{13}C_3$]serine

^{13}C-Excess (mol%) of polar metabolites from experiments with *Fth* WT, *Fth* LVS and *Fno* grown in medium T supplemented with 3 mM [U-$^{13}C_3$]serine. Mean and SD from three independent experiments are shown

3 mM [U-$^{13}C_3$]serine	*Fth* WT	*Fth* LVS	*Fno*
Lactate	0.12% ± 0.01%	0.12% ± 0.01%	5.70% ± 0.44%

Glycolic acid	0.41% ± 0.17%	0.31% ± 0.12%	0.06% ± 0.06%
Alanine	0.85% ± 0.03%	0.96% ± 0.08%	5.73% ± 0.38%
Glycine	1.67% ± 0.01%	1.27% ± 0.02%	8.48% ± 0.13%
Oxalic acid	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
3-hydroxy butyric acid	3.02% ± 0.17%	2.40% ± 0.09%	0.85% ± 0.07%
Valine	0.09% ± 0.03%	0.08% ± 0.04%	0.09% ± 0.03%
leucine	0.05% ± 0.01%	0.05% ± 0.01%	0.06% ± 0.02%
Isoleucine	0.14% ± 0.04%	0.15% ± 0.04%	0.14% ± 0.05%
Succinic acid	0.21% ± 0.03%	0.23% ± 0.07%	0.21% ± 0.00%
Fumaric acid	0.18% ± 0.06%	0.28% ± 0.26%	0.36% ± 0.15%
Glycerol	0.26% ± 0.22%	0.27% ± 0.21%	0.34% ± 0.16%
Methionine	0.22% ± 0.08%	0.16% ± 0.04%	0.12% ± 0.01%
Serine	9.58% ± 0.88%	5.71% ± 0.08%	20.38% ± 0.60%
Threonine	0.14% ± 0.08%	0.17% ± 0.00%	0.19% ± 0.11%
Phenylalanine	0.17% ± 0.04%	0.13% ± 0.01%	0.28% ± 0.11%
Malic acid	0.92% ± 0.24%	2.10% ± 0.93%	2.56% ± 0.18%
Aspartate	0.11% ± 0.05%	0.08% ± 0.08%	0.14% ± 0.06%
Glutamate	0.51% ± 0.05%	0.39% ± 0.00%	0.27% ± 0.01%
Palmitic acid	5.05% ± 0.54%	5.30% ± 0.36%	0.88% ± 0.31%
Oleic acid	8.97% ± 1.02%	8.66% ± 0.49%	3.75% ± 0.99%
Stearic acid	2.86% ± 0.08%	2.69% ± 0.12%	1.29% ± 0.55%
Citric acid	2.91% ± 0.00%	0.12% ± 0.01%	0.38% ± 0.11%

Supplemental Table S8 ^{13}C-Excess (mol%) of polar metabolites from [U-$^{13}C_3$] glycerol

^{13}C-Excess (mol%) of polar metabolites from experiments with *Fth* WT, *Fth* LVS and *Fno* grown in medium T supplemented with 25 mM [U-$^{13}C_3$] glycerol. Mean and SD from three independent experiments are shown.

25 mM [U-$^{13}C_3$]glycerol	*Fth* WT	*Fth* LVS	*Fno*
Lactate	1.21% ± 0.07%	1.32% ± 0.08%	11.27% ± 4.89%
Glycolic acid	6.95% ± 4.99%	2.29% ± 0.12%	6.24% ± 3.82%

Alanine	4.74% ± 1.70%	2.52% ± 0.34%	17.51% ± 5.98%
Glycine	1.40% ± 1.35%	0.11% ± 0.05%	1.65% ± 1.71%
Oxalic acid	2.06% ± 2.06%	0.05% ± 0.05%	0.81% ± 1.11%
3-hydroxy butyric acid	5.63% ± 1.76%	4.35% ± 1.02%	28.43% ± 3.28%
Valine	0.19% ± 0.16%	0.05% ± 0.03%	0.21% ± 0.18%
leucine	0.04% ± 0.04%	0.01% ± 0.01%	0.03% ± 0.01%
Isoleucine	0.14% ± 0.10%	0.07% ± 0.03%	0.15% ± 0.05%
Succinic acid	7.79% ± 6.82%	1.98% ± 0.46%	13.50% ± 3.71%
Fumaric acid	4.83% ± 4.32%	0.51% ± 0.38%	6.75% ± 4.12%
Glycerol	92.98% ± 0.11%	92.70% ± 0.57%	58.52% ± 26.17%
Methionine	0.18% ± 0.18%	0.10% ± 0.10%	0.11% ± 0.08%
Serine	0.63% ± 0.20%	0.36% ± 0.10%	0.53% ± 0.30%
Threonine	0.25% ± 0.06%	0.18% ± 0.12%	0.26% ± 0.09%
Phenylalanine	0.75% ± 0.10%	1.15% ± 0.05%	0.19% ± 0.05%
Malic acid	4.89% ± 2.55%	5.42% ± 0.71%	19.81% ± 2.53%
Aspartate	0.55% ± 0.43%	0.29% ± 0.13%	0.47% ± 0.34%
Glutamate	3.10% ± 1.18%	1.88% ± 0.22%	7.47% ± 1.86%
Palmitic acid	2.28% ± 2.03%	1.54% ± 0.81%	13.93% ± 6.23%
Oleic acid	3.53% ± 0.92%	3.31% ± 0.19%	16.48% ± 3.55%
Stearic acid	2.83% ± 2.50%	1.41% ± 0.82%	12.90% ± 7.23%
Citric acid	1.28% ± 0.88%	1.08% ± 0.23%	5.15% ± 5.58%

Supplemental Table S9 ^{13}C-Excess (mol%) of sugars from [U-$^{13}C_6$]glucose

^{13}C-Excess (mol%) of sugars from experiments with *Fth* WT, *Fth* LVS and *Fno* grown in medium T supplemented with 11 mM [U-$^{13}C_6$]glucose. Mean and SD from three independent experiments are shown.

11 mM [U-$^{13}C_6$]glucose	*Fth* WT	*Fth* LVS	*Fno*
Glucose in glycogen	10.41% ± 0.44%	10.60% ± 0.54%	9.68% ± 0.94%
Free fructose	12.48% ± 0.77%	11.71% ± 0.54%	12.18% ± 0.39%
Glucosamine	10.77% ± 1.23%	10.16% ± 0.87%	10.22% ± 0.95%

Muramic acid	12.19% ± 1.55%	11.92% ± 1.52%	10.05% ± 1.44%

11 mM [U-$^{13}C_6$]glucose	*Fth* WT and *Fth* LVS	*Fno*
averaged over the four hexoses	11.16% ± 0.90%	10.53% ± 1.12%

Supplemental Table S10 ^{13}C-Excess (mol%) of sugars from [1,2-$^{13}C_2$]glucose

^{13}C-Excess (mol%) of sugars from experiments with *Fth* WT grown in medium T supplemented with 11 mM [1,2-$^{13}C_2$]glucose. Mean and SD from two independent experiments are shown.

11 mM [1,2-$^{13}C_2$]glucose	*Fth* WT	*Fth* LVS	*Fno*
Glucose in glycogen	4.55% ± 0.09%		
Free fructose	4.89% ± 0.14%		
Glucosamine	5.25% ± 0.01%		
Muramic acid	7.85% ± 0.03%		

Supplemental Table S11 ^{13}C-Excess (mol%) of sugars from [U-$^{13}C_3$]serine

^{13}C-Excess (mol%) of sugars from experiments with *Fth* WT, *Fth* LVS and *Fno* grown in medium T supplemented with 3 mM [U-$^{13}C_3$]serine. Mean and SD from three independent experiments are shown.

3 mM [U-$^{13}C_3$]serine	*Fth* WT	*Fth* LVS	*Fno*
Glucose in glycogen	0.06% ± 0.05%	0.05% ± 0.04%	0.02% ± 0.02%
Free fructose	0.55% ± 0.04%	0.53% ± 0.00%	0.46% ± 0.00%
Glucosamine	0.42% ± 0.11%	0.31% ± 0.04%	0.39% ± 0.01%
Muramic acid	0.66% ± 0.14%	0.59% ± 0.04%	1.83% ± 0.69%

Supplemental Table S12 ^{13}C-Excess (mol%) of sugars from [U-$^{13}C_3$] glycerol

^{13}C-Excess (mol%) of sugars from experiments with *Fth* WT, *Fth* LVS and *Fno* grown in medium T supplemented with 25 mM [U-$^{13}C_3$] glycerol. Mean and SD from three independent experiments are shown.

25 mM [U-$^{13}C_3$]glycerol	*Fth* WT	*Fth* LVS	*Fno*
Glucose in glycogen	0.58% ± 0.04%	0.42% ± 0.04%	1.07% ± 0.22%
Free fructose	0.80% ± 0.05%	0.59% ± 0.11%	1.46% ± 0.16%
Glucosamine	1.34% ± 0.20%	0.95% ± 0.08%	3.46% ± 0.28%

Muramic acid	4.98% ± 0.46%	3.55% ± 0.17%	14.93% ± 8.25%

Supplemental Table S13 Relative fractions of isotopologues (mol%) of amino acids from experiments with *Fth* WT, *Fth* LVS and *Fno* grown in medium T supplemented with 11 mM [U-$^{13}C_6$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

11 mM [U-$^{13}C_6$]glucose *Fth* WT								
	Alanine	Aspartate	Glutamate	Glycine	Phenylalanine	Serine	Threonine	Tyrosine
M+1	1.80 % ± 0.30 %	2.51 % ± 0.64 %	5.63 % ± 0.45 %	0.53 % ± 0.20 %	1.17 % ± 0.37 %	0.03 % ± 0.08 %	1.78 % ± 0.33 %	0.18 % ± 0.39 %
M+2	1.33 % ± 0.06 %	1.61 % ± 0.40 %	5.60 % ± 0.32 %	0.09 % ± 0.07 %	0.01 % ± 0.02 %	0.00 % ± 0.00 %	0.37 % ± 0.13 %	0.97 % ± 0.19 %
M+3	6.34 % ± 0.35 %	0.33 % ± 0.07 %	0.61 % ± 0.08 %		0.12 % ± 0.06 %	0.16 % ± 0.04 %	0.00 % ± 0.00 %	1.00 % ± 0.09 %
M+4		0.00 % ± 0.00 %	0.18 % ± 0.04 %		0.25 % ± 0.02 %		0.00 % ± 0.00 %	0.54 % ± 0.05 %
M+5			0.03 % ± 0.00 %		0.03 % ± 0.02 %			0.26 % ± 0.03 %
M+6					0.00 % ± 0.00 %			0.11 % ± 0.03 %
M+7					0.00 % ± 0.00 %			0.00 % ± 0.01 %
M+8					0.00 % ± 0.00 %			0.01 % ± 0.01 %
M+9					0.00 % ± 0.00 %			0.08 % ± 0.05 %

11 mM [U-$^{13}C_6$]glucose *Fth* LVS								
	Alanine	Aspartate	Glutamate	Glycine	Phenylalanine	Serine	Threonine	Tyrosine
M+1	1.94 % ± 0.48 %	2.62 % ± 1.32 %	5.31 % ± 0.57 %	0.20 % ± 0.21 %	0.66 % ± 0.50 %	0.00 % ± 0.00 %	1.68 % ± 0.58 %	0.19 % ± 0.30 %
M+2	1.24 % ± 0.24 %	1.26 % ± 0.72 %	5.72 % ± 0.49 %	0.03 % ± 0.06 %	0.05 % ± 0.08 %	0.00 % ± 0.00 %	0.32 % ± 0.27 %	0.56 % ± 0.26 %

M+3	6.40 % ± 0.36 %	0.28 % ± 0.09 %	0.54 % ± 0.09 %		0.30 % ± 0.12 %	0.13 % ± 0.06 %	0.00 % ± 0.00 %	0.67 % ± 0.11 %
M+4		0.00 % ± 0.01 %	0.15 % ± 0.05 %		0.38 % ± 0.04 %		0.00 % ± 0.00 %	0.44 % ± 0.09 %
M+5			0.02 % ± 0.01 %		0.06 % ± 0.02 %			0.25 % ± 0.04 %
M+6					0.00 % ± 0.00 %			0.06 % ± 0.04 %
M+7					0.00 % ± 0.00 %			0.00 % ± 0.01 %
M+8					0.00 % ± 0.00 %			0.02 % ± 0.01 %
M+9					0.00 % ± 0.00 %			0.01 % ± 0.02 %

11 mM [U-$^{13}C_6$]glucose *Fno*								
	Alanine	Aspartate	Glutamate	Glycine	Phenylalanine	Serine	Threonine	Tyrosine
M+1	1.26 % ± 0.39 %	1.22 % ± 0.49 %	4.74 % ± 1.18 %	0.57 % ± 0.20 %	0.62 % ± 0.45 %	0.61 % ± 0.51 %	1.79 % ± 0.66 %	0.35 % ± 0.39 %
M+2	1.06 % ± 0.22 %	0.55 % ± 0.36 %	6.65 % ± 0.76 %	0.55 % ± 0.15 %	0.00 % ± 0.00 %	0.16 % ± 0.17 %	0.42 % ± 0.23 %	1.99 % ± 0.33 %
M+3	7.92 % ± 0.65 %	0.18 % ± 0.14 %	0.61 % ± 0.17 %		0.00 % ± 0.00 %	0.56 % ± 0.12 %	0.00 % ± 0.00 %	2.17 % ± 0.22 %
M+4		0.00 % ± 0.00 %	0.19 % ± 0.10 %		0.14 % ± 0.02 %		0.00 % ± 0.00 %	1.65 % ± 0.21 %
M+5			0.02 % ± 0.01 %		0.00 % ± 0.01 %			0.43 % ± 0.06 %
M+6					0.00 % ± 0.00 %			0.28 % ± 0.05 %
M+7					0.00 % ± 0.00 %			0.10 % ± 0.06 %
M+8					0.04 % ± 0.06 %			0.01 % ± 0.02 %

M+9					0.00 % ± 0.00 %			0.02 % ± 0.05 %

Supplemental Table S14 Relative fractions of isotopologues (mol%) of amino acids from experiments with *Fth* WT, *Fth* LVS and *Fno* grown in medium T supplemented with 11 mM [1,2-$^{13}C_2$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

11 mM [1,2-$^{13}C_2$]glucose *Fth* WT								
	Alanine	Aspartate	Glutamate	Glycine	Phenylalanine	Serine	Threonine	Tyrosine
M+1	0.72 % ± 0.34 %	0.98 % ± 0.42 %	2.60 % ± 0.54 %	0.29 % ± 0.11 %	1.35 % ± 0.39 %	0.00 % ± 0.00 %	1.69 % ± 0.27 %	0.00 % ± 0.00 %
M+2	3.49 % ± 0.19 %	0.47 % ± 0.09 %	2.55 % ± 0.24 %	0.17 % ± 0.02 %	0.08 % ± 0.09 %	0.00 % ± 0.00 %	0.55 % ± 0.10 %	1.58 % ± 0.27 %
M+3	0.06 % ± 0.02 %	0.12 % ± 0.06 %	0.11 % ± 0.07 %		0.00 % ± 0.00 %	0.21 % ± 0.03 %	0.00 % ± 0.00 %	0.08 % ± 0.10 %
M+4		0.00 % ± 0.00 %	0.00 % ± 0.00 %		0.13 % ± 0.02 %		0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+5			0.01 % ± 0.01 %		0.00 % ± 0.00 %			0.06 % ± 0.01 %
M+6					0.00 % ± 0.00 %			0.01 % ± 0.02 %
M+7					0.00 % ± 0.00 %			0.00 % ± 0.00 %
M+8					0.00 % ± 0.00 %			0.00 % ± 0.00 %
M+9					0.00 % ± 0.00 %			0.01 % ± 0.02 %

11 mM [1,2-$^{13}C_2$]glucose *Fth* LVS								
	Alanine	Aspartate	Glutamate	Glycine	Phenylalanine	Serine	Threonine	Tyrosine

M+1	1.54 % ± 0.09 %	1.35 % ± 0.54 %	3.51 % ± 0.24 %	0.01 % ± 0.02 %	0.00 % ± 0.00 %	0.13 % ± 0.20 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+2	4.33 % ± 0.17 %	1.28 % ± 0.19 %	3.78 % ± 0.32 %	0.02 % ± 0.02 %	0.42 % ± 0.11 %	0.50 % ± 0.10 %	0.00 % ± 0.01 %	1.17 % ± 0.23 %
M+3	0.04 % ± 0.02 %	0.04 % ± 0.03 %	0.17 % ± 0.08 %		0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.17 % ± 0.07 %	0.14 % ± 0.14 %
M+4		0.00 % ± 0.00 %	0.06 % ± 0.01 %		0.01 % ± 0.01 %		0.00 % ± 0.00 %	0.09 % ± 0.15 %
M+5			0.00 % ± 0.00 %		0.04 % ± 0.03 %			0.07 % ± 0.12 %
M+6					0.02 % ± 0.02 %			0.08 % ± 0.10 %
M+7					0.01 % ± 0.01 %			0.04 % ± 0.09 %
M+8					0.01 % ± 0.02 %			0.10 % ± 0.14 %
M+9					0.02 % ± 0.02 %			0.08 % ± 0.16 %

11 mM [1,2-$^{13}C_2$]glucose *Fno*								
	Alanine	Aspartate	Glutamate	Glycine	Phenylalanine	Serine	Threonine	Tyrosine
M+1	0.98 % ± 0.12 %	0.32 % ± 0.16 %	3.14 % ± 0.18 %	0.38 % ± 0.11 %	0.00 % ± 0.00 %	2.71 % ± 0.31 %	0.00 % ± 0.00 %	0.62 % ± 0.59 %
M+2	4.84 % ± 0.09 %	0.71 % ± 0.12 %	4.31 % ± 0.11 %	0.00 % ± 0.01 %	0.48 % ± 0.32 %	0.56 % ± 0.16 %	0.78 % ± 0.14 %	3.23 % ± 0.36 %
M+3	0.08 % ± 0.06 %	0.02 % ± 0.03 %	0.20 % ± 0.08 %		0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.26 % ± 0.30 %
M+4		0.02 % ± 0.02 %	0.08 % ± 0.04 %		0.02 % ± 0.03 %		0.05 % ± 0.08 %	0.00 % ± 0.00 %
M+5			0.01 % ± 0.01 %		0.09 % ± 0.11 %			0.23 % ± 0.26 %
M+6					0.02 % ± 0.02 %			0.11 % ± 0.18 %

M+7					0.08 % ± 0.13 %			0.15 % ± 0.18 %
M+8					0.08 % ± 0.09 %			0.00 % ± 0.01 %
M+9					0.04 % ± 0.08 %			0.52 % ± 0.63 %

Supplemental Table S15 Relative fractions of isotopologues (mol%) of amino acids from experiments with *Fth* WT, *Fth* LVS and *Fno* grown in medium T supplemented with 3 mM [U-$^{13}C_3$]serine. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

3 mM [U-$^{13}C_3$]serine *Fth* WT								
	Alanine	Aspartate	Glutamate	Glycine	Phenylalanine	Serine	Threonine	Tyrosine
M+1	1.07 % ± 0.32 %	2.09 % ± 0.28 %	2.95 % ± 0.26 %	0.66 % ± 0.14 %	0.47 % ± 0.35 %	0.92 % ± 0.24 %	1.60 % ± 0.32 %	0.08 % ± 0.20 %
M+2	0.64 % ± 0.06 %	0.79 % ± 0.09 %	3.38 % ± 0.17 %	3.35 % ± 0.22 %	0.00 % ± 0.00 %	0.84 % ± 0.07 %	0.27 % ± 0.15 %	0.20 % ± 0.15 %
M+3	4.34 % ± 0.06 %	0.10 % ± 0.02 %	0.13 % ± 0.09 %		0.00 % ± 0.00 %	12.06 % ± 0.11 %	0.00 % ± 0.00 %	0.01 % ± 0.02 %
M+4		0.00 % ± 0.00 %	0.05 % ± 0.03 %		0.12 % ± 0.02 %		0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+5			0.03 % ± 0.01 %		0.00 % ± 0.00 %			0.08 % ± 0.03 %
M+6					0.00 % ± 0.00 %			0.00 % ± 0.00 %
M+7					0.00 % ± 0.00 %			0.00 % ± 0.00 %
M+8					0.00 % ± 0.00 %			0.00 % ± 0.00 %
M+9					0.00 % ± 0.00 %			0.52 % ± 0.58 %

3 mM [U-$^{13}C_3$]serine *Fth* LVS								
	Alanine	Aspartate	Glutamate	Glycine	Phenylalanine	Serine	Threonine	Tyrosine
M+1	0.80 % ± 0.45 %	2.05 % ± 0.37 %	3.31 % ± 0.28 %	0.50 % ± 0.18 %	0.16 % ± 0.14 %	0.15 % ± 0.31 %	1.46 % ± 0.28 %	0.02 % ± 0.04 %
M+2	0.43 % ± 0.18 %	1.13 % ± 0.11 %	4.11 % ± 0.18 %	2.43 % ± 0.24 %	0.00 % ± 0.00 %	0.36 % ± 0.12 %	0.43 % ± 0.17 %	0.11 % ± 0.09 %
M+3	5.23 % ± 0.21 %	0.15 % ± 0.04 %	0.30 % ± 0.10 %		0.00 % ± 0.00 %	12.10 % ± 0.24 %	0.00 % ± 0.00 %	0.01 % ± 0.02 %
M+4		0.00 % ± 0.00 %	0.12 % ± 0.03 %		0.13 % ± 0.03 %		0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+5			0.03 % ± 0.01 %		0.00 % ± 0.00 %			0.09 % ± 0.04 %
M+6					0.00 % ± 0.00 %			0.00 % ± 0.01 %
M+7					0.00 % ± 0.00 %			0.00 % ± 0.00 %
M+8					0.00 % ± 0.00 %			0.00 % ± 0.00 %
M+9					0.00 % ± 0.00 %			0.08 % ± 0.11 %

3 mM [U-$^{13}C_3$]serine *Fno*								
	Alanine	Aspartate	Glutamate	Glycine	Phenylalanine	Serine	Threonine	Tyrosine
M+1	0.45 % ± 0.33 %	0.57 % ± 0.37 %	0.74 % ± 0.37 %	1.35 % ± 0.18 %	0.01 % ± 0.01 %	5.83 % ± 0.64 %	1.19 % ± 0.25 %	0.00 % ± 0.01 %
M+2	0.23 % ± 0.09 %	0.00 % ± 0.00 %	0.55 % ± 0.05 %	7.61 % ± 0.66 %	0.02 % ± 0.04 %	3.42 % ± 0.27 %	0.43 % ± 0.29 %	0.24 % ± 0.13 %
M+3	1.14 % ± 0.04 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %		0.00 % ± 0.00 %	11.80 % ± 0.31 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %

M+4		0.00 % ± 0.00 %	0.00 % ± 0.00 %		0.02 % ± 0.01 %		0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+5			0.00 % ± 0.00 %		0.00 % ± 0.00 %			0.08 % ± 0.03 %
M+6					0.00 % ± 0.00 %			0.00 % ± 0.00 %
M+7					0.00 % ± 0.00 %			0.00 % ± 0.00 %
M+8					0.00 % ± 0.00 %			0.00 % ± 0.00 %
M+9					0.12 % ± 0.04 %			0.27 % ± 0.32 %

Supplemental Table S16 Relative fractions of isotopologues (mol%) of amino acids from experiments with *Fth* WT, *Fth* LVS and *Fno* grown in medium T supplemented with 25 mM [U-$^{13}C_3$]glycerol. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

25 mM [U-$^{13}C_3$]glycerol *Fth* WT								
	Alanine	Aspartate	Glutamate	Glycine	Phenylalanine	Serine	Threonine	Tyrosine
M+1	0.74 % ± 0.11 %	2.66 % ± 0.43 %	3.66 % ± 0.56 %	0.43 % ± 0.17 %	0.24 % ± 0.28 %	0.18 % ± 0.20 %	1.21 % ± 0.29 %	0.29 % ± 0.42 %
M+2	0.34 % ± 0.06 %	0.82 % ± 0.20 %	3.54 % ± 0.81 %	0.03 % ± 0.04 %	0.09 % ± 0.13 %	0.00 % ± 0.00 %	0.31 % ± 0.24 %	0.73 % ± 0.14 %
M+3	4.32 % ± 1.03 %	0.12 % ± 0.09 %	0.29 % ± 0.15 %		0.15 % ± 0.14 %	0.11 % ± 0.05 %	0.00 % ± 0.00 %	0.75 % ± 0.05 %
M+4		0.00 % ± 0.00 %	0.06 % ± 0.07 %		0.14 % ± 0.01 %		0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+5			0.02 % ± 0.01 %		0.02 % ± 0.02 %			0.19 % ± 0.03 %
M+6					0.00 % ± 0.00 %			0.00 % ± 0.01 %
M+7					0.00 % ± 0.00 %			0.00 % ± 0.00 %

M+8					0.00 % ± 0.00 %			0.00 % ± 0.00 %
M+9					0.00 % ± 0.00 %			0.00 % ± 0.00 %

25 mM [U-$^{13}C_3$]glycerol *Fth* LVS								
	Alanine	Aspartate	Glutamate	Glycine	Phenylalanine	Serine	Threonine	Tyrosine
M+1	0.51 % ± 0.34 %	1.52 % ± 0.96 %	2.52 % ± 0.95 %	0.22 % ± 0.13 %	0.17 % ± 0.20 %	0.01 % ± 0.03 %	0.79 % ± 0.50 %	0.06 % ± 0.14 %
M+2	0.16 % ± 0.11 %	0.36 % ± 0.19 %	3.13 % ± 0.17 %	0.03 % ± 0.04 %	0.02 % ± 0.03 %	0.00 % ± 0.00 %	0.06 % ± 0.07 %	0.74 % ± 0.31 %
M+3	3.23 % ± 0.16 %	0.11 % ± 0.11 %	0.08 % ± 0.05 %		0.55 % ± 0.07 %	0.15 % ± 0.05 %	0.00 % ± 0.00 %	0.51 % ± 0.21 %
M+4		0.00 % ± 0.00 %	0.01 % ± 0.01 %		0.17 % ± 0.05 %		0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+5			0.02 % ± 0.01 %		0.05 % ± 0.10 %			0.13 % ± 0.02 %
M+6					0.02 % ± 0.04 %			0.00 % ± 0.00 %
M+7					0.00 % ± 0.01 %			0.00 % ± 0.00 %
M+8					0.00 % ± 0.01 %			0.00 % ± 0.00 %
M+9					0.00 % ± 0.00 %			0.00 % ± 0.00 %

25 mM [U-$^{13}C_3$]glycerol *Fno*								
	Alanine	Aspartate	Glutamate	Glycine	Phenylalanine	Serine	Threonine	Tyrosine
M+1	1.24 % ± 0.35 %	1.49 % ± 0.96 %	6.71 % ± 0.91 %	1.05 % ± 0.21 %	0.13 % ± 0.19 %	1.68 % ± 0.66 %	1.15 % ± 0.71 %	0.37 % ± 0.53 %

M+2	1.29 % ± 0.22 %	0.97 % ± 0.53 %	13.09 % ± 1.62 %	1.77 % ± 0.46 %	0.03 % ± 0.08 %	0.79 % ± 0.30 %	0.41 % ± 0.51 %	4.95 % ± 0.29 %
M+3	21.27 % ± 2.36 %	0.40 % ± 0.19 %	2.50 % ± 0.76 %		0.00 % ± 0.00 %	1.47 % ± 0.61 %	0.00 % ± 0.00 %	5.64 % ± 0.62 %
M+4		0.03 % ± 0.04 %	1.22 % ± 0.47 %		0.11 % ± 0.04 %		0.00 % ± 0.00 %	0.14 % ± 0.11 %
M+5			0.20 % ± 0.09 %		0.03 % ± 0.05 %			2.96 % ± 0.36 %
M+6					0.01 % ± 0.03 %			0.42 % ± 0.16 %
M+7					0.00 % ± 0.01 %			0.02 % ± 0.05 %
M+8					0.11 % ± 0.12 %			0.10 % ± 0.07 %
M+9					0.01 % ± 0.01 %			0.01 % ± 0.02 %

Supplemental Table S17 Relative fractions of isotopologues (mol%) of polar metabolites from experiments with *Fth* WT, *Fth* LVS and *Fno* grown in medium T supplemented with 11 mM [U-$^{13}C_6$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

11 mM [U-$^{13}C_6$]glucose *Fth* WT								
	Glycolic acid	Oxalate	Succinate	Fumarate	Malate	Aspartate	Glutamate	Citrate
M+1	0.65 % ± 0.19 %	0.22 % ± 0.15 %	4.88 % ± 0.66 %	0.59 % ± 0.91 %	11.30 % ± 2.55 %	0.75 % ± 0.21 %	8.71 % ± 0.38 %	0.03 % ± 0.08 %
M+2	10.06 % ± 0.75 %	2.44 % ± 0.26 %	3.68 % ± 0.36 %	0.71 % ± 0.96 %	4.24 % ± 1.17 %	0.06 % ± 0.09 %	8.81 % ± 0.41 %	0.17 % ± 0.46 %
M+3			0.89 % ± 0.08 %	0.53 % ± 0.19 %	1.06 % ± 1.03 %	0.25 % ± 0.05 %	1.03 % ± 0.12 %	0.04 % ± 0.09 %
M+4			0.00 % ± 0.01 %	0.18 % ± 0.20 %	0.26 % ± 0.51 %	0.01 % ± 0.02 %	0.33 % ± 0.03 %	0.36 % ± 0.61 %

M+5							0.06 % ± 0.01 %	0.41 % ± 0.80 %
M+6								0.11 % ± 0.22 %

11 mM [U-$^{13}C_6$]glucose *Fth* WT							
	Lactic acid	Alanine	Glycine	Glycerol	Serine	Threonine	Phenylalanine
M+1	0.00 % ± 0.00 %	2.82 % ± 0.18 %	0.43 % ± 0.05 %	1.82 % ± 0.33 %	0.47 % ± 0.28 %	0.01 % ± 0.03 %	0.44 % ± 0.27 %
M+2	0.55 % ± 0.09 %	1.96 % ± 0.04 %	0.05 % ± 0.06 %	1.08 % ± 0.10 %	0.48 % ± 0.17 %	0.29 % ± 0.09 %	0.71 % ± 0.18 %
M+3	6.95 % ± 0.84 %	7.71 % ± 0.53 %		6.83 % ± 0.43 %	0.06 % ± 0.06 %	0.03 % ± 0.06 %	1.12 % ± 0.15 %
M+4						0.03 % ± 0.04 %	0.76 % ± 0.08 %
M+5							0.20 % ± 0.02 %
M+6							0.10 % ± 0.01 %
M+7							0.06 % ± 0.01 %
M+8							0.00 % ± 0.00 %
M+9							0.00 % ± 0.00 %

11 mM [U-$^{13}C_6$]glucose *Fth* WT				
	3-Hydroxybutyric acid	Palmitic acid	Oleic acid	Stearic acid
M+1	2.63 % ± 0.73 %	2.55 % ± 0.27 %	5.34 % ± 0.33 %	3.80 % ± 0.18 %

M+2	15.58 % ± 1.28 %	22.35 % ± 0.70 %	24.63 % ± 1.17 %	22.79 % ± 0.99 %
M+3	0.43 % ± 0.62 %	1.78 % ± 0.06 %	2.78 % ± 0.16 %	2.87 % ± 0.18 %
M+4	0.99 % ± 0.52 %	7.67 % ± 0.38 %	7.75 % ± 0.67 %	10.22 % ± 0.98 %
M+5		0.55 % ± 0.09 %	1.01 % ± 0.31 %	1.19 % ± 0.25 %
M+6		1.94 % ± 0.48 %	2.20 % ± 0.88 %	3.45 % ± 1.27 %
M+7		0.12 % ± 0.08 %	0.38 % ± 0.19 %	0.41 % ± 0.26 %
M+8		0.41 % ± 0.30 %	0.63 % ± 0.54 %	1.09 % ± 0.90 %
M+9		0.03 % ± 0.04 %	0.09 % ± 0.09 %	0.14 % ± 0.15 %
M+10		0.07 % ± 0.11 %	0.20 % ± 0.18 %	0.36 % ± 0.41 %
M+11		0.00 % ± 0.01 %	0.03 % ± 0.03 %	0.04 % ± 0.05 %
M+12		0.02 % ± 0.03 %	0.04 % ± 0.05 %	0.07 % ± 0.12 %
M+13		0.04 % ± 0.02 %	0.01 % ± 0.01 %	0.01 % ± 0.01 %
M+14		0.01 % ± 0.01 %	0.02 % ± 0.02 %	0.01 % ± 0.02 %
M+15		0.01 % ± 0.01 %	0.01 % ± 0.01 %	0.01 % ± 0.01 %
M+16		0.00 % ± 0.01 %	0.02 % ± 0.02 %	0.00 % ± 0.00 %
M+17			0.01 % ± 0.01 %	0.04 % ± 0.03 %
M+18			0.01 % ± 0.01 %	0.01 % ± 0.01 %

11 mM [U-$^{13}C_6$]glucose *Fth* LVS								
	Glycolic acid	Oxalate	Succinate	Fumarate	Malate	Aspartate	Glutamate	Citrate
M+1	0.91 % ± 0.32 %	0.32 % ± 0.21 %	9.03 % ± 2.33 %	0.73 % ± 0.71 %	0.31 % ± 0.74 %	0.60 % ± 0.53 %	6.23 % ± 1.36 %	1.10 % ± 1.41 %
M+2	8.86 % ± 1.31 %	1.92 % ± 0.30 %	5.21 % ± 0.71 %	1.05 % ± 0.95 %	0.73 % ± 1.34 %	0.17 % ± 0.18 %	6.46 % ± 0.74 %	0.38 % ± 0.43 %
M+3			1.05 % ± 0.12 %	0.63 % ± 0.21 %	0.04 % ± 0.16 %	0.15 % ± 0.12 %	0.88 % ± 0.20 %	0.02 % ± 0.04 %
M+4			0.01 % ± 0.02 %	0.44 % ± 0.37 %	0.30 % ± 0.60 %	0.01 % ± 0.02 %	0.27 % ± 0.08 %	0.34 % ± 0.52 %
M+5							0.03 % ± 0.02 %	0.12 % ± 0.41 %
M+6								0.37 % ± 0.51 %

11 mM [U-$^{13}C_6$]glucose *Fth* LVS							
	Lactic acid	Alanine	Glycine	Glycerol	Serine	Threonine	Phenylalanine
M+1	0.07 % ± 0.11 %	2.56 % ± 0.50 %	0.15 % ± 0.18 %	1.38 % ± 0.36 %	0.31 % ± 0.26 %	0.00 % ± 0.02 %	0.54 % ± 0.37 %
M+2	0.88 % ± 0.19 %	1.90 % ± 0.27 %	0.12 % ± 0.19 %	0.85 % ± 0.18 %	0.47 % ± 0.31 %	0.24 % ± 0.29 %	1.76 % ± 0.34 %
M+3	7.06 % ± 1.36 %	7.77 % ± 0.47 %		6.61 % ± 0.83 %	0.04 % ± 0.06 %	0.03 % ± 0.06 %	2.14 % ± 0.36 %
M+4						0.02 % ± 0.04 %	1.44 % ± 0.16 %
M+5							0.35 % ± 0.05 %
M+6							0.25 % ± 0.03 %
M+7							0.14 % ± 0.02 %

M+8							0.00 % ± 0.00 %
M+9							0.00 % ± 0.00 %

11 mM [U-$^{13}C_6$]glucose *Fth* LVS				
	3-Hydroxybutyric acid	Palmitic acid	Oleic acid	Stearic acid
M+1	2.38 % ± 0.52 %	1.95 % ± 1.23 %	4.87 % ± 0.67 %	3.55 % ± 0.79 %
M+2	16.83 % ± 1.01 %	20.46 % ± 4.96 %	26.35 % ± 1.13 %	23.29 % ± 3.87 %
M+3	0.21 % ± 0.17 %	1.54 % ± 0.53 %	2.78 % ± 0.47 %	2.66 % ± 0.75 %
M+4	0.99 % ± 0.21 %	6.60 % ± 1.75 %	8.58 % ± 0.49 %	9.99 % ± 1.90 %
M+5		0.40 % ± 0.16 %	0.85 % ± 0.14 %	1.01 % ± 0.34 %
M+6		1.38 % ± 0.44 %	2.01 % ± 0.14 %	2.80 % ± 0.64 %
M+7		0.07 % ± 0.03 %	0.18 % ± 0.07 %	0.25 % ± 0.13 %
M+8		0.18 % ± 0.11 %	0.36 % ± 0.10 %	0.61 % ± 0.30 %
M+9		0.01 % ± 0.01 %	0.03 % ± 0.02 %	0.05 % ± 0.06 %
M+10		0.01 % ± 0.03 %	0.05 % ± 0.05 %	0.13 % ± 0.14 %
M+11		0.00 % ± 0.00 %	0.00 % ± 0.01 %	0.01 % ± 0.02 %
M+12		0.00 % ± 0.00 %	0.01 % ± 0.02 %	0.02 % ± 0.04 %
M+13		0.04 % ± 0.05 %	0.00 % ± 0.01 %	0.00 % ± 0.01 %

M+14		0.00 % ± 0.01 %	0.00 % ± 0.01 %	0.02 % ± 0.01 %
M+15		0.00 % ± 0.01 %	0.00 % ± 0.01 %	0.01 % ± 0.01 %
M+16		0.00 % ± 0.00 %	0.01 % ± 0.01 %	0.00 % ± 0.01 %
M+17			0.00 % ± 0.00 %	0.03 % ± 0.03 %
M+18			0.00 % ± 0.00 %	0.01 % ± 0.01 %

11 mM [U-$^{13}C_6$]glucose *Fno*								
	Glycolic acid	Oxalate	Succinate	Fumarate	Malate	Aspartate	Glutamate	Citrate
M+1	0.57 % ± 0.40 %	0.15 % ± 0.24 %	8.60 % ± 2.44 %	0.84 % ± 0.88 %	5.25 % ± 6.66 %	0.21 % ± 0.26 %	4.16 % ± 0.91 %	0.35 % ± 0.69 %
M+2	9.07 % ± 1.81 %	1.49 % ± 0.47 %	6.22 % ± 0.97 %	1.26 % ± 1.04 %	2.91 % ± 3.83 %	0.02 % ± 0.06 %	5.33 % ± 0.47 %	0.68 % ± 1.12 %
M+3			1.29 % ± 0.32 %	0.62 % ± 0.31 %	1.98 % ± 2.53 %	0.03 % ± 0.07 %	0.58 % ± 0.10 %	0.30 % ± 0.41 %
M+4			0.01 % ± 0.01 %	0.64 % ± 0.31 %	0.38 % ± 0.91 %	0.00 % ± 0.00 %	0.17 % ± 0.06 %	0.08 % ± 0.14 %
M+5							0.03 % ± 0.01 %	0.04 % ± 0.08 %
M+6								0.13 % ± 0.38 %

11 mM [U-$^{13}C_6$]glucose *Fno*							
	Lactic acid	Alanine	Glycine	Glycerol	Serine	Threonine	Phenylalanine
M+1	0.18 % ± 0.24 %	1.12 % ± 0.33 %	0.25 % ± 0.21 %	1.15 % ± 0.44 %	0.53 % ± 0.40 %	0.08 % ± 0.17 %	0.08 % ± 0.24 %

M+2	0.86 % ± 0.16 %	1.15 % ± 0.17 %	0.55 % ± 0.25 %	1.01 % ± 0.40 %	0.51 % ± 0.31 %	0.29 % ± 0.19 %	0.00 % ± 0.01 %
M+3	7.17 % ± 0.59 %	8.86 % ± 0.77 %		8.23 % ± 1.23 %	0.04 % ± 0.08 %	0.01 % ± 0.03 %	0.02 % ± 0.03 %
M+4						0.01 % ± 0.02 %	0.22 % ± 0.01 %
M+5							0.06 % ± 0.02 %
M+6							0.00 % ± 0.00 %
M+7							0.01 % ± 0.01 %
M+8							0.05 % ± 0.06 %
M+9							0.00 % ± 0.00 %

11 mM [U-$^{13}C_6$]glucose *Fno*				
	3-Hydroxybutyric acid	Palmitic acid	Oleic acid	Stearic acid
M+1	1.82 % ± 0.47 %	1.41 % ± 0.62 %	4.30 % ± 0.24 %	2.84 % ± 0.29 %
M+2	19.85 % ± 1.00 %	23.29 % ± 3.84 %	28.33 % ± 0.96 %	26.31 % ± 2.63 %
M+3	0.27 % ± 0.13 %	1.23 % ± 0.21 %	2.64 % ± 0.26 %	2.32 % ± 0.23 %
M+4	1.30 % ± 0.20 %	8.27 % ± 1.37 %	10.21 % ± 0.90 %	12.24 % ± 1.20 %
M+5		0.32 % ± 0.07 %	0.84 % ± 0.09 %	0.92 % ± 0.12 %
M+6		1.82 % ± 0.36 %	2.52 % ± 0.37 %	3.44 % ± 0.49 %
M+7		0.05 % ± 0.02 %	0.17 % ± 0.05 %	0.22 % ± 0.06 %

M+8		0.24 % ± 0.08 %	0.45 % ± 0.10 %	0.67 % ± 0.16 %
M+9		0.01 % ± 0.01 %	0.03 % ± 0.01 %	0.03 % ± 0.02 %
M+10		0.01 % ± 0.01 %	0.06 % ± 0.03 %	0.10 % ± 0.06 %
M+11		0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+12		0.00 % ± 0.00 %	0.01 % ± 0.01 %	0.00 % ± 0.00 %
M+13		0.02 % ± 0.01 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+14		0.00 % ± 0.00 %	0.01 % ± 0.01 %	0.00 % ± 0.01 %
M+15		0.01 % ± 0.01 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+16		0.00 % ± 0.00 %	0.00 % ± 0.01 %	0.00 % ± 0.00 %
M+17			0.00 % ± 0.01 %	0.00 % ± 0.01 %
M+18			0.00 % ± 0.01 %	0.00 % ± 0.00 %

Supplemental Table S18 Relative fractions of isotopologues (mol%) of polar metabolites from experiments with *Fth* WT grown in medium T supplemented with 11 mM [1,2-$^{13}C_2$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from two independent experiments are shown.

11 mM [1,2-$^{13}C_2$]glucose *Fth* WT								
	Glycolic acid	Oxalate	Succinate	Fumarate	Malate	Aspartate	Glutamate	Citrate
M+1	0.94 % ± 0.30 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.78 % ± 0.88 %	0.53 % ± 0.31 %	3.63 % ± 0.29 %	0.00 % ± 0.00 %
M+2	4.58 % ± 0.26 %	0.20 % ± 0.15 %	0.74 % ± 0.22 %	0.15 % ± 0.16 %	1.24 % ± 0.33 %	0.00 % ± 0.00 %	3.16 % ± 0.42 %	0.52 % ± 1.22 %

M+3			0.37 % ± 0.02 %	0.34 % ± 0.33 %	0.29 % ± 0.45 %	0.08 % ± 0.05 %	0.01 % ± 0.02 %	1.10 % ± 1.49 %
M+4			0.01 % ± 0.01 %	0.02 % ± 0.05 %	0.18 % ± 0.24 %	0.00 % ± 0.00 %	0.02 % ± 0.01 %	0.23 % ± 0.50 %
M+5							0.03 % ± 0.01 %	0.06 % ± 0.14 %
M+6								0.34 % ± 0.52 %

11 mM [1,2-$^{13}C_2$]glucose *Fth* WT							
	Lactic acid	Alanine	Glycine	Glycerol	Serine	Threonine	Phenylalanine
M+1	0.00 % ± 0.00 %	1.10 % ± 0.16 %	0.22 % ± 0.10 %	0.74 % ± 0.25 %	0.38 % ± 0.22 %	0.00 % ± 0.00 %	0.30 % ± 0.20 %
M+2	7.50 % ± 0.61 %	4.00 % ± 0.33 %	0.02 % ± 0.03 %	3.55 % ± 0.37 %	0.40 % ± 0.13 %	0.31 % ± 0.12 %	1.42 % ± 0.26 %
M+3	0.04 % ± 0.04 %	0.12 % ± 0.01 %		0.12 % ± 0.04 %	0.04 % ± 0.06 %	0.02 % ± 0.04 %	0.03 % ± 0.02 %
M+4						0.01 % ± 0.01 %	0.24 % ± 0.01 %
M+5							0.03 % ± 0.01 %
M+6							0.00 % ± 0.00 %
M+7							0.00 % ± 0.00 %
M+8							0.00 % ± 0.00 %
M+9							0.00 % ± 0.00 %

11 mM [1,2-$^{13}C_2$]glucose *Fth* WT

	3-Hydroxybutyric acid	Palmitic acid	Oleic acid	Stearic acid
M+1	1.25 % ± 0.39 %	0.89 % ± 0.22 %	3.79 % ± 0.35 %	3.58 % ± 0.34 %
M+2	5.66 % ± 0.83 %	15.27 % ± 1.20 %	18.24 % ± 1.48 %	18.86 % ± 1.36 %
M+3	0.13 % ± 0.18 %	0.51 % ± 0.12 %	1.17 % ± 0.28 %	1.36 % ± 0.33 %
M+4	0.09 % ± 0.19 %	2.53 % ± 0.53 %	2.97 % ± 0.74 %	4.20 % ± 0.92 %
M+5		0.08 % ± 0.03 %	0.21 % ± 0.11 %	0.34 % ± 0.13 %
M+6		0.30 % ± 0.13 %	0.43 % ± 0.21 %	0.73 % ± 0.29 %
M+7		0.02 % ± 0.01 %	0.04 % ± 0.02 %	0.05 % ± 0.04 %
M+8		0.02 % ± 0.03 %	0.07 % ± 0.04 %	0.13 % ± 0.07 %
M+9		0.01 % ± 0.01 %	0.01 % ± 0.01 %	0.01 % ± 0.01 %
M+10		0.00 % ± 0.00 %	0.05 % ± 0.03 %	0.03 % ± 0.02 %
M+11		0.00 % ± 0.00 %	0.01 % ± 0.01 %	0.00 % ± 0.00 %
M+12		0.00 % ± 0.00 %	0.01 % ± 0.01 %	0.00 % ± 0.00 %
M+13		0.04 % ± 0.02 %	0.01 % ± 0.01 %	0.00 % ± 0.00 %
M+14		0.00 % ± 0.00 %	0.01 % ± 0.01 %	0.02 % ± 0.02 %
M+15		0.02 % ± 0.01 %	0.01 % ± 0.00 %	0.01 % ± 0.01 %
M+16		0.00 % ± 0.00 %	0.01 % ± 0.02 %	0.01 % ± 0.01 %
M+17			0.01 % ± 0.02 %	0.03 % ± 0.01 %

M+18			0.02 % ± 0.02 %	0.01 % ± 0.01 %

Supplemental Table S19 Relative fractions of isotopologues (mol%) of polar metabolites from experiments with *Fth* WT, *Fth* LVS and *Fno* grown in medium T supplemented with 3 mM [U-$^{13}C_3$]serine. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

3 mM [U-$^{13}C_3$]serine *Fth* WT								
	Glycolic acid	Oxalate	Succinate	Fumarate	Malate	Aspartate	Glutamate	Citrate
M+1	0.06 % ± 0.11 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.78 % ± 0.93 %	0.38 % ± 0.39 %	0.92 % ± 0.34 %	0.09 % ± 0.22 %
M+2	0.34 % ± 0.23 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	1.38 % ± 0.53 %	0.00 % ± 0.00 %	0.81 % ± 0.05 %	0.03 % ± 0.06 %
M+3			0.20 % ± 0.03 %	0.42 % ± 0.22 %	0.00 % ± 0.00 %	0.02 % ± 0.04 %	0.00 % ± 0.01 %	0.18 % ± 0.45 %
M+4			0.01 % ± 0.01 %	0.04 % ± 0.09 %	0.80 % ± 1.11 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+5							0.01 % ± 0.01 %	0.13 % ± 0.25 %
M+6								0.24 % ± 0.52 %

3 mM [U-$^{13}C_3$]serine *Fth* WT							
	Lactic acid	Alanine	Glycine	Glycerol	Serine	Threonine	Phenylalanine
M+1	0.00 % ± 0.00 %	0.39 % ± 0.20 %	0.46 % ± 0.20 %	0.62 % ± 0.71 %	0.03 % ± 0.08 %	0.38 % ± 0.50 %	0.42 % ± 0.34 %
M+2	0.01 % ± 0.02 %	0.13 % ± 0.05 %	1.44 % ± 0.03 %	0.03 % ± 0.04 %	0.62 % ± 0.21 %	0.01 % ± 0.02 %	0.00 % ± 0.00 %
M+3	0.11 % ± 0.05 %	0.63 % ± 0.04 %		0.04 % ± 0.01 %	9.15 % ± 0.96 %	0.02 % ± 0.03 %	0.00 % ± 0.00 %

M+4						0.02 % ± 0.03 %	0.11 % ± 0.03 %
M+5							0.07 % ± 0.05 %
M+6							0.02 % ± 0.03 %
M+7							0.03 % ± 0.04 %
M+8							0.00 % ± 0.00 %
M+9							0.00 % ± 0.00 %

3 mM [U-$^{13}C_3$]serine *Fth* WT				
	3-Hydroxybutyric acid	Palmitic acid	Oleic acid	Stearic acid
M+1	2.00 % ± 0.27 %	2.59 % ± 0.57 %	6.70 % ± 0.26 %	4.43 % ± 0.47 %
M+2	3.49 % ± 0.45 %	13.23 % ± 1.33 %	21.11 % ± 0.54 %	9.79 % ± 1.81 %
M+3	0.21 % ± 0.29 %	2.56 % ± 0.48 %	5.19 % ± 1.17 %	1.49 % ± 0.08 %
M+4	0.50 % ± 0.27 %	5.09 % ± 0.74 %	9.87 % ± 2.16 %	2.30 % ± 0.23 %
M+5		1.20 % ± 0.27 %	2.81 % ± 0.91 %	0.51 % ± 0.08 %
M+6		1.65 % ± 0.34 %	3.82 % ± 1.19 %	0.63 % ± 0.08 %
M+7		0.34 % ± 0.08 %	0.99 % ± 0.35 %	0.15 % ± 0.06 %
M+8		0.37 % ± 0.07 %	1.06 % ± 0.36 %	0.15 % ± 0.04 %
M+9		0.05 % ± 0.02 %	0.22 % ± 0.09 %	0.02 % ± 0.02 %

M+10		0.04 % ± 0.01 %	0.18 % ± 0.06 %	0.01 % ± 0.01 %
M+11		0.01 % ± 0.02 %	0.01 % ± 0.01 %	0.01 % ± 0.01 %
M+12		0.05 % ± 0.08 %	0.02 % ± 0.02 %	0.03 % ± 0.05 %
M+13		0.04 % ± 0.03 %	0.01 % ± 0.03 %	0.01 % ± 0.01 %
M+14		0.01 % ± 0.02 %	0.03 % ± 0.03 %	0.11 % ± 0.17 %
M+15		0.01 % ± 0.02 %	0.01 % ± 0.01 %	0.05 % ± 0.07 %
M+16		0.01 % ± 0.01 %	0.01 % ± 0.01 %	0.02 % ± 0.04 %
M+17			0.00 % ± 0.00 %	0.07 % ± 0.04 %
M+18			0.01 % ± 0.01 %	0.03 % ± 0.05 %

3 mM [U-$^{13}C_3$]serine *Fth* LVS								
	Glycolic acid	Oxalate	Succinate	Fumarate	Malate	Aspartate	Glutamate	Citrate
M+1	0.07 % ± 0.16 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	1.04 % ± 1.61 %	0.21 % ± 0.33 %	0.57 % ± 0.34 %	0.05 % ± 0.13 %
M+2	0.30 % ± 0.24 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	1.56 % ± 2.82 %	0.00 % ± 0.00 %	0.67 % ± 0.15 %	0.09 % ± 0.21 %
M+3			0.18 % ± 0.05 %	0.42 % ± 0.30 %	1.16 % ± 1.48 %	0.02 % ± 0.05 %	0.00 % ± 0.00 %	0.60 % ± 1.46 %
M+4			0.00 % ± 0.00 %	0.11 % ± 0.18 %	1.12 % ± 1.05 %	0.01 % ± 0.03 %	0.00 % ± 0.00 %	0.32 % ± 0.55 %
M+5							0.01 % ± 0.01 %	0.13 % ± 0.32 %
M+6								0.48 % ± 1.19 %

3 mM [U-$^{13}C_3$]serine *Fth* LVS							
	Lactic acid	Alanine	Glycine	Glycerol	Serine	Threonine	Phenylalanine
M+1	0.00 % ± 0.00 %	0.30 % ± 0.15 %	0.26 % ± 0.08 %	0.60 % ± 0.73 %	0.02 % ± 0.05 %	0.41 % ± 0.38 %	0.32 % ± 0.19 %
M+2	0.00 % ± 0.00 %	0.10 % ± 0.03 %	1.15 % ± 0.05 %	0.03 % ± 0.04 %	0.33 % ± 0.23 %	0.05 % ± 0.12 %	0.00 % ± 0.00 %
M+3	0.12 % ± 0.03 %	0.80 % ± 0.06 %		0.04 % ± 0.05 %	5.48 % ± 0.23 %	0.05 % ± 0.06 %	0.05 % ± 0.06 %
M+4						0.01 % ± 0.02 %	0.14 % ± 0.01 %
M+5							0.04 % ± 0.02 %
M+6							0.00 % ± 0.00 %
M+7							0.00 % ± 0.00 %
M+8							0.00 % ± 0.00 %
M+9							0.00 % ± 0.00 %

3 mM [U-$^{13}C_3$]serine *Fth* LVS				
	3-Hydroxybutyric acid	Palmitic acid	Oleic acid	Stearic acid
M+1	1.10 % ± 0.44 %	2.20 % ± 0.28 %	5.70 % ± 0.44 %	4.09 % ± 0.43 %
M+2	3.86 % ± 0.46 %	14.56 % ± 0.80 %	20.30 % ± 0.58 %	9.97 % ± 1.48 %
M+3	0.01 % ± 0.02 %	2.07 % ± 0.16 %	4.32 % ± 0.44 %	1.23 % ± 0.16 %
M+4	0.41 % ± 0.50 %	5.61 % ± 0.55 %	9.71 % ± 1.33 %	2.24 % ± 0.36 %

M+5		1.01 % ± 0.16 %	2.34 % ± 0.41 %	0.42 % ± 0.05 %
M+6		1.98 % ± 0.26 %	4.01 % ± 0.82 %	0.67 % ± 0.07 %
M+7		0.31 % ± 0.04 %	0.92 % ± 0.21 %	0.11 % ± 0.02 %
M+8		0.47 % ± 0.08 %	1.22 % ± 0.28 %	0.15 % ± 0.03 %
M+9		0.05 % ± 0.02 %	0.23 % ± 0.07 %	0.01 % ± 0.01 %
M+10		0.06 % ± 0.02 %	0.22 % ± 0.07 %	0.01 % ± 0.02 %
M+11		0.02 % ± 0.02 %	0.04 % ± 0.02 %	0.01 % ± 0.01 %
M+12		0.02 % ± 0.03 %	0.03 % ± 0.02 %	0.02 % ± 0.03 %
M+13		0.03 % ± 0.02 %	0.01 % ± 0.01 %	0.01 % ± 0.01 %
M+14		0.00 % ± 0.00 %	0.02 % ± 0.02 %	0.07 % ± 0.12 %
M+15		0.00 % ± 0.01 %	0.01 % ± 0.02 %	0.04 % ± 0.04 %
M+16		0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.02 % ± 0.02 %
M+17			0.00 % ± 0.01 %	0.04 % ± 0.03 %
M+18			0.01 % ± 0.01 %	0.03 % ± 0.03 %

3 mM [U-$^{13}C_3$]serine *Fno*								
	Glycolic acid	Oxalate	Succinate	Fumarate	Malate	Aspartate	Glutamate	Citrate
M+1	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.97 % ± 2.36 %	0.33 % ± 0.30 %	0.32 % ± 0.25 %	0.00 % ± 0.00 %

M+2	0.09 % ± 0.08 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	2.64 % ± 4.52 %	0.00 % ± 0.00 %	0.50 % ± 0.08 %	0.10 % ± 0.25 %
M+3			0.26 % ± 0.01 %	0.88 % ± 0.85 %	0.36 % ± 0.81 %	0.03 % ± 0.05 %	0.01 % ± 0.02 %	0.12 % ± 0.30 %
M+4			0.01 % ± 0.01 %	0.06 % ± 0.11 %	3.99 % ± 6.61 %	0.03 % ± 0.06 %	0.00 % ± 0.00 %	0.42 % ± 0.79 %
M+5							0.00 % ± 0.00 %	0.17 % ± 0.30 %
M+6								0.00 % ± 0.00 %

3 mM [U-$^{13}C_3$]serine *Fno*							
	Lactic acid	Alanine	Glycine	Glycerol	Serine	Threonine	Phenylalanine
M+1	0.90 % ± 0.19 %	1.14 % ± 0.08 %	0.84 % ± 0.10 %	0.82 % ± 0.47 %	0.22 % ± 0.16 %	0.50 % ± 0.63 %	0.25 % ± 0.15 %
M+2	1.23 % ± 0.08 %	0.85 % ± 0.03 %	8.06 % ± 0.15 %	0.04 % ± 0.10 %	0.99 % ± 0.21 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+3	4.58 % ± 0.45 %	4.78 % ± 0.44 %		0.04 % ± 0.06 %	19.65 % ± 0.54 %	0.04 % ± 0.05 %	0.02 % ± 0.05 %
M+4						0.03 % ± 0.03 %	0.14 % ± 0.02 %
M+5							0.07 % ± 0.05 %
M+6							0.02 % ± 0.03 %
M+7							0.00 % ± 0.00 %
M+8							0.14 % ± 0.11 %
M+9							0.00 % ± 0.00 %

3 mM [U-$^{13}C_3$]serine *Fno*				
	3-Hydroxybutyric acid	Palmitic acid	Oleic acid	Stearic acid
M+1	0.99 % ± 0.17 %	0.14 % ± 0.25 %	3.83 % ± 0.77 %	2.87 % ± 0.82 %
M+2	1.18 % ± 0.13 %	4.20 % ± 1.58 %	11.92 % ± 3.52 %	5.55 % ± 3.02 %
M+3	0.00 % ± 0.00 %	0.30 % ± 0.16 %	1.65 % ± 0.58 %	0.57 % ± 0.35 %
M+4	0.00 % ± 0.00 %	0.48 % ± 0.32 %	3.69 % ± 1.42 %	0.98 % ± 0.70 %
M+5		0.06 % ± 0.05 %	0.82 % ± 0.38 %	0.17 % ± 0.11 %
M+6		0.10 % ± 0.07 %	1.36 % ± 0.48 %	0.25 % ± 0.14 %
M+7		0.02 % ± 0.03 %	0.31 % ± 0.12 %	0.03 % ± 0.03 %
M+8		0.00 % ± 0.00 %	0.41 % ± 0.14 %	0.05 % ± 0.03 %
M+9		0.01 % ± 0.02 %	0.06 % ± 0.03 %	0.01 % ± 0.02 %
M+10		0.00 % ± 0.00 %	0.04 % ± 0.04 %	0.01 % ± 0.01 %
M+11		0.00 % ± 0.01 %	0.00 % ± 0.00 %	0.01 % ± 0.01 %
M+12		0.01 % ± 0.02 %	0.01 % ± 0.01 %	0.00 % ± 0.00 %
M+13		0.06 % ± 0.01 %	0.01 % ± 0.01 %	0.00 % ± 0.00 %
M+14		0.01 % ± 0.02 %	0.02 % ± 0.02 %	0.00 % ± 0.00 %
M+15		0.03 % ± 0.02 %	0.02 % ± 0.02 %	0.00 % ± 0.00 %
M+16		0.01 % ± 0.01 %	0.03 % ± 0.03 %	0.00 % ± 0.00 %

M+17			0.01 % ± 0.01 %	0.01 % ± 0.01 %
M+18			0.01 % ± 0.02 %	0.01 % ± 0.02 %

Supplemental Table S20 Relative fractions of isotopologues (mol%) of polar metabolites from experiments with *Fth* WT, *Fth* LVS and *Fno* grown in medium T supplemented with 25 mM [U-$^{13}C_3$]glycerol. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

25 mM [U-$^{13}C_3$]glycerol *Fth* WT								
	Glycolic acid	Oxalate	Succinate	Fumarate	Malate	Aspartate	Glutamate	Citrate
M+1	1.37 % ± 1.44 %	1.71 % ± 1.89 %	4.23 % ± 3.95 %	12.38 % ± 13.57 %	8.87 % ± 6.87 %	1.68 % ± 1.66 %	4.92 % ± 2.40 %	1.30 % ± 2.02 %
M+2	5.42 % ± 3.84 %	0.28 % ± 0.31 %	9.19 % ± 8.99 %	2.31 % ± 1.95 %	2.38 % ± 1.29 %	0.03 % ± 0.06 %	4.56 % ± 1.57 %	0.58 % ± 1.39 %
M+3			1.10 % ± 0.80 %	0.34 % ± 0.36 %	0.31 % ± 0.64 %	0.14 % ± 0.10 %	0.27 % ± 0.27 %	0.14 % ± 0.31 %
M+4			0.28 % ± 0.27 %	0.11 % ± 0.21 %	0.30 % ± 0.38 %	0.01 % ± 0.02 %	0.12 % ± 0.04 %	0.19 % ± 0.45 %
M+5							0.03 % ± 0.02 %	0.37 % ± 0.58 %
M+6								0.21 % ± 0.35 %

25 mM [U-$^{13}C_3$]glycerol *Fth* WT							
	Lactic acid	Alanine	Glycine	Glycerol	Serine	Threonine	Phenylalanine
M+1	0.03 % ± 0.08 %	1.19 % ± 0.74 %	2.21 % ± 2.41 %	0.05 % ± 0.01 %	0.82 % ± 0.52 %	0.33 % ± 0.50 %	0.26 % ± 0.20 %
M+2	0.14 % ± 0.21 %	0.31 % ± 0.15 %	0.29 % ± 0.28 %	2.44 % ± 0.09 %	0.47 % ± 0.27 %	0.22 % ± 0.19 %	0.57 % ± 0.19 %

M+3	1.11 % ± 0.14 %	4.13 % ± 1.55 %		91.33 % ± 0.20 %	0.05 % ± 0.06 %	0.04 % ± 0.09 %	1.10 % ± 0.15 %
M+4						0.03 % ± 0.05 %	0.14 % ± 0.02 %
M+5							0.22 % ± 0.08 %
M+6							0.06 % ± 0.06 %
M+7							0.01 % ± 0.01 %
M+8							0.00 % ± 0.00 %
M+9							0.00 % ± 0.00 %

25 mM [U-$^{13}C_3$]glycerol *Fth* WT				
	3-Hydroxybutyric acid	Palmitic acid	Oleic acid	Stearic acid
M+1	1.75 % ± 0.93 %	0.67 % ± 0.73 %	3.67 % ± 1.30 %	2.37 % ± 1.21 %
M+2	7.97 % ± 4.36 %	10.32 % ± 9.83 %	17.73 % ± 3.14 %	11.86 % ± 11.49 %
M+3	0.87 % ± 0.70 %	0.48 % ± 0.40 %	1.26 % ± 0.43 %	0.84 % ± 0.85 %
M+4	0.68 % ± 0.65 %	2.56 % ± 2.55 %	3.43 % ± 1.27 %	3.59 % ± 3.64 %
M+5		0.09 % ± 0.09 %	0.27 % ± 0.14 %	0.26 % ± 0.26 %
M+6		0.42 % ± 0.46 %	0.58 % ± 0.33 %	0.76 % ± 0.79 %
M+7		0.01 % ± 0.02 %	0.04 % ± 0.03 %	0.04 % ± 0.04 %
M+8		0.05 % ± 0.05 %	0.08 % ± 0.07 %	0.12 % ± 0.13 %

M+9		0.00 % ± 0.00 %	0.01 % ± 0.01 %	0.01 % ± 0.01 %
M+10		0.00 % ± 0.00 %	0.02 % ± 0.02 %	0.02 % ± 0.02 %
M+11		0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+12		0.00 % ± 0.00 %	0.01 % ± 0.01 %	0.00 % ± 0.00 %
M+13		0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+14		0.00 % ± 0.00 %	0.00 % ± 0.01 %	0.00 % ± 0.00 %
M+15		0.01 % ± 0.01 %	0.01 % ± 0.01 %	0.00 % ± 0.00 %
M+16		0.00 % ± 0.00 %	0.01 % ± 0.03 %	0.00 % ± 0.00 %
M+17			0.00 % ± 0.01 %	0.01 % ± 0.01 %
M+18			0.02 % ± 0.03 %	0.02 % ± 0.03 %

25 mM [U-$^{13}C_3$]glycerol *Fth* LVS								
	Glycolic acid	Oxalate	Succinate	Fumarate	Malate	Aspartate	Glutamate	Citrate
M+1	0.19 % ± 0.19 %	0.00 % ± 0.00 %	2.52 % ± 0.93 %	0.00 % ± 0.00 %	2.55 % ± 4.34 %	0.62 % ± 0.58 %	2.37 % ± 0.36 %	1.22 % ± 1.93 %
M+2	2.20 % ± 0.43 %	0.05 % ± 0.12 %	2.04 % ± 0.43 %	0.43 % ± 0.59 %	0.02 % ± 0.05 %	0.11 % ± 0.28 %	3.28 % ± 0.55 %	0.03 % ± 0.07 %
M+3			0.41 % ± 0.11 %	0.25 % ± 0.37 %	1.06 % ± 1.18 %	0.06 % ± 0.08 %	0.08 % ± 0.11 %	0.51 % ± 0.66 %
M+4			0.02 % ± 0.03 %	0.11 % ± 0.21 %	1.27 % ± 1.49 %	0.03 % ± 0.05 %	0.03 % ± 0.03 %	0.11 % ± 0.28 %
M+5							0.03 % ± 0.02 %	0.39 % ± 0.96 %

M+6								0.22 % ± 0.35 %

25 mM [U-$^{13}C_3$]glycerol *Fth* LVS							
	Lactic acid	Alanine	Glycine	Glycerol	Serine	Threonine	Phenylalanine
M+1	0.00 % ± 0.00 %	0.31 % ± 0.20 %	0.15 % ± 0.15 %	0.05 % ± 0.03 %	0.23 % ± 0.40 %	0.00 % ± 0.00 %	0.11 % ± 0.19 %
M+2	0.08 % ± 0.11 %	0.11 % ± 0.08 %	0.03 % ± 0.04 %	2.44 % ± 0.04 %	0.10 % ± 0.24 %	0.11 % ± 0.17 %	1.38 % ± 0.19 %
M+3	1.26 % ± 0.15 %	2.34 % ± 0.36 %		91.06 % ± 0.64 %	0.22 % ± 0.27 %	0.14 % ± 0.21 %	1.99 % ± 0.08 %
M+4						0.02 % ± 0.04 %	0.09 % ± 0.03 %
M+5							0.19 % ± 0.08 %
M+6							0.03 % ± 0.04 %
M+7							0.00 % ± 0.00 %
M+8							0.00 % ± 0.00 %
M+9							0.00 % ± 0.00 %

25 mM [U-$^{13}C_3$]glycerol *Fth* LVS				
	3-Hydroxybutyric acid	Palmitic acid	Oleic acid	Stearic acid
M+1	1.36 % ± 1.23 %	0.11 % ± 0.23 %	2.82 % ± 0.33 %	0.81 % ± 0.80 %
M+2	6.34 % ± 1.90 %	8.67 % ± 5.13 %	18.80 % ± 1.06 %	7.44 % ± 4.87 %

M+3	0.44 % ± 0.61 %	0.23 % ± 0.10 %	0.89 % ± 0.17 %	0.40 % ± 0.24 %
M+4	0.51 % ± 0.67 %	1.42 % ± 0.87 %	3.15 % ± 0.25 %	1.62 % ± 1.13 %
M+5		0.03 % ± 0.02 %	0.16 % ± 0.07 %	0.06 % ± 0.04 %
M+6		0.10 % ± 0.08 %	0.39 % ± 0.05 %	0.19 % ± 0.13 %
M+7		0.00 % ± 0.00 %	0.02 % ± 0.01 %	0.01 % ± 0.01 %
M+8		0.00 % ± 0.00 %	0.04 % ± 0.02 %	0.02 % ± 0.01 %
M+9		0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+10		0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+11		0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+12		0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+13		0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.01 % ± 0.01 %
M+14		0.00 % ± 0.00 %	0.01 % ± 0.01 %	0.00 % ± 0.00 %
M+15		0.00 % ± 0.00 %	0.00 % ± 0.01 %	0.01 % ± 0.00 %
M+16		0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.01 %
M+17			0.00 % ± 0.00 %	0.01 % ± 0.01 %
M+18			0.00 % ± 0.00 %	0.00 % ± 0.00 %

25 mM [U-$^{13}C_3$]glycerol *Fno*

	Glycolic acid	Oxalate	Succinate	Fumarate	Malate	Aspartate	Glutamate	Citrate
M+1	1.50 % ± 1.97 %	1.12 % ± 1.61 %	12.89 % ± 2.33 %	9.50 % ± 10.42 %	10.01 % ± 9.01 %	1.24 % ± 1.45 %	5.64 % ± 1.64 %	2.23 % ± 1.81 %
M+2	5.00 % ± 2.37 %	0.28 % ± 0.42 %	12.23 % ± 3.10 %	4.43 % ± 3.17 %	7.84 % ± 6.44 %	0.04 % ± 0.13 %	9.21 % ± 1.65 %	0.58 % ± 0.94 %
M+3			4.80 % ± 1.87 %	2.10 % ± 1.41 %	1.60 % ± 2.29 %	0.10 % ± 0.14 %	2.45 % ± 0.83 %	2.71 % ± 6.45 %
M+4			0.73 % ± 0.66 %	0.87 % ± 0.90 %	5.38 % ± 7.05 %	0.06 % ± 0.11 %	1.16 % ± 0.49 %	1.05 % ± 2.19 %
M+5							0.25 % ± 0.15 %	0.39 % ± 0.75 %
M+6								2.64 % ± 5.83 %

25 mM [U-$^{13}C_3$]glycerol *Fno*							
	Lactic acid	Alanine	Glycine	Glycerol	Serine	Threonine	Phenylalanine
M+1	0.23 % ± 0.30 %	1.12 % ± 0.91 %	1.39 % ± 1.64 %	0.22 % ± 0.22 %	0.54 % ± 0.65 %	0.00 % ± 0.00 %	0.18 % ± 0.24 %
M+2	0.74 % ± 0.45 %	1.10 % ± 0.62 %	0.95 % ± 1.00 %	1.44 % ± 0.81 %	0.32 % ± 0.29 %	0.40 % ± 0.29 %	0.00 % ± 0.01 %
M+3	10.70 % ± 4.81 %	16.40 % ± 5.66 %		57.49 % ± 27.26 %	0.14 % ± 0.13 %	0.06 % ± 0.06 %	0.05 % ± 0.06 %
M+4						0.02 % ± 0.02 %	0.15 % ± 0.02 %
M+5							0.09 % ± 0.04 %
M+6							0.00 % ± 0.00 %
M+7							0.00 % ± 0.01 %

M+8							0.04 % ± 0.03 %
M+9							0.00 % ± 0.00 %

25 mM [U-$^{13}C_3$]glycerol *Fno*				
	3-Hydroxybutyric acid	Palmitic acid	Oleic acid	Stearic acid
M+1	2.80 % ± 0.49 %	1.16 % ± 0.88 %	3.28 % ± 0.33 %	1.28 % ± 0.55 %
M+2	38.28 % ± 2.61 %	15.24 % ± 5.09 %	19.76 % ± 0.75 %	11.15 % ± 4.40 %
M+3	1.16 % ± 0.52 %	2.96 % ± 1.36 %	4.87 % ± 1.25 %	2.04 % ± 0.99 %
M+4	7.70 % ± 1.84 %	14.61 % ± 6.33 %	16.51 % ± 4.03 %	11.45 % ± 5.94 %
M+5		2.43 % ± 1.23 %	4.17 % ± 1.32 %	2.21 % ± 1.27 %
M+6		9.16 % ± 4.57 %	10.70 % ± 3.41 %	9.13 % ± 5.59 %
M+7		1.20 % ± 0.65 %	2.28 % ± 0.84 %	1.54 % ± 1.03 %
M+8		3.88 % ± 2.26 %	4.93 % ± 1.94 %	5.15 % ± 3.52 %
M+9		0.38 % ± 0.24 %	0.87 % ± 0.37 %	0.75 % ± 0.56 %
M+10		1.10 % ± 0.77 %	1.61 % ± 0.74 %	2.07 % ± 1.60 %
M+11		0.03 % ± 0.04 %	0.21 % ± 0.11 %	0.25 % ± 0.22 %
M+12		0.17 % ± 0.15 %	0.38 % ± 0.21 %	0.57 % ± 0.49 %
M+13		0.01 % ± 0.01 %	0.04 % ± 0.03 %	0.05 % ± 0.05 %

M+14		0.01 % ± 0.02 %	0.07 % ± 0.05 %	0.08 % ± 0.08 %
M+15		0.01 % ± 0.02 %	0.00 % ± 0.01 %	0.00 % ± 0.00 %
M+16		0.00 % ± 0.00 %	0.01 % ± 0.02 %	0.00 % ± 0.00 %
M+17			0.00 % ± 0.01 %	0.00 % ± 0.00 %
M+18			0.02 % ± 0.02 %	0.00 % ± 0.00 %

Supplemental Table S21 Relative fractions of isotopologues (mol%) of sugars from experiments with *Fth* WT, *Fth* LVS and *Fno* grown in medium T supplemented with 11 mM [U-$^{13}C_6$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

11 mM [U-$^{13}C_6$]glucose *Fth* WT				
	Glucose in glycogen	Free fructose	Muramic acid	Glucosamine
M+1	0.98 % ± 0.24 %	0.13 % ± 0.21 %	4.91 % ± 2.33 %	2.80 % ± 0.72 %
M+2	1.30 % ± 0.17 %	0.37 % ± 0.61 %	4.72 % ± 1.97 %	3.37 % ± 0.52 %
M+3	1.73 % ± 0.21 %	1.23 % ± 0.28 %	7.49 % ± 1.67 %	2.49 % ± 0.66 %
M+4	0.70 % ± 0.07 %	0.74 % ± 0.32 %	3.42 % ± 0.88 %	1.35 % ± 0.17 %
M+5	0.57 % ± 0.05 %	0.05 % ± 0.07 %	0.35 % ± 0.54 %	1.10 % ± 0.10 %
M+6	8.00 % ± 0.72 %	11.18 % ± 0.96 %	3.49 % ± 1.84 %	6.12 % ± 0.92 %

11 mM [U-$^{13}C_6$]glucose *Fth* LVS				
	Glucose in glycogen	Free fructose	Muramic acid	Glucosamine
M+1	2.58 % ± 2.36 %	0.04 % ± 0.14 %	6.21 % ± 2.44 %	3.87 % ± 2.34 %
M+2	1.74 % ± 0.19 %	0.39 % ± 0.43 %	4.58 % ± 2.74 %	3.02 % ± 0.50 %
M+3	1.98 % ± 0.31 %	1.09 % ± 0.70 %	7.45 % ± 2.61 %	1.97 % ± 0.60 %
M+4	1.01 % ± 0.06 %	0.38 % ± 0.43 %	3.47 % ± 2.07 %	1.36 % ± 0.17 %
M+5	0.54 % ± 0.04 %	0.04 % ± 0.08 %	0.54 % ± 1.03 %	1.05 % ± 0.29 %
M+6	7.48 % ± 0.62 %	10.74 % ± 0.63 %	2.87 % ± 1.61 %	5.75 % ± 0.78 %

11 mM [U-$^{13}C_6$]glucose *Fno*				
	Glucose in glycogen	Free fructose	Muramic acid	Glucosamine
M+1	0.16 % ± 0.23 %	0.30 % ± 0.65 %	5.47 % ± 3.92 %	3.17 % ± 2.26 %
M+2	0.61 % ± 0.24 %	1.02 % ± 0.83 %	2.87 % ± 2.45 %	2.81 % ± 0.75 %
M+3	0.61 % ± 0.19 %	1.70 % ± 0.71 %	4.66 % ± 3.53 %	1.69 % ± 0.70 %
M+4	0.41 % ± 0.12 %	0.93 % ± 0.47 %	3.18 % ± 1.87 %	1.47 % ± 0.35 %
M+5	0.52 % ± 0.12 %	0.22 % ± 0.36 %	0.14 % ± 0.44 %	0.98 % ± 0.31 %
M+6	8.44 % ± 0.88 %	10.13 % ± 0.54 %	3.62 % ± 1.44 %	6.69 % ± 0.97 %

Supplemental Table S22 Relative fractions of isotopologues (mol%) of sugars from experiments with *Fth* WT grown in medium T supplemented with 11 mM [1,2-$^{13}C_2$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from two independent experiments are shown.

11 mM [1,2-$^{13}C_2$]glucose *Fth* WT				
	Glucose in glycogen	Free fructose	Muramic acid	Glucosamine
M+1	0.24 % ± 0.24 %	0.00 % ± 0.00 %	2.19 % ± 1.94 %	1.46 % ± 0.88 %
M+2	13.30 % ± 0.52 %	11.31 % ± 0.78 %	18.83 % ± 1.09 %	14.15 % ± 0.55 %
M+3	0.01 % ± 0.01 %	0.66 % ± 0.73 %	0.00 % ± 0.01 %	0.02 % ± 0.07 %
M+4	0.08 % ± 0.03 %	1.19 % ± 0.28 %	1.55 % ± 0.33 %	0.01 % ± 0.02 %
M+5	0.00 % ± 0.01 %	0.00 % ± 0.00 %	0.12 % ± 0.18 %	0.33 % ± 0.08 %
M+6	0.01 % ± 0.01 %	0.00 % ± 0.00 %	0.07 % ± 0.11 %	0.00 % ± 0.00 %

Supplemental Table S23 Relative fractions of isotopologues (mol%) of sugars from experiments with *Fth* WT, *Fth* LVS and *Fno* grown in medium T supplemented with 3 mM [U-$^{13}C_3$]serine. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

3 mM [U-$^{13}C_3$]serine *Fth* WT				
	Glucose in glycogen	Free fructose	Muramic acid	Glucosamine
M+1	0.01 % ± 0.03 %	0.00 % ± 0.01 %	1.25 % ± 1.60 %	1.09 % ± 0.93 %
M+2	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.61 % ± 0.53 %	0.48 % ± 0.38 %
M+3	0.00 % ± 0.00 %	0.89 % ± 0.24 %	0.05 % ± 0.11 %	0.12 % ± 0.18 %
M+4	0.00 % ± 0.00 %	0.16 % ± 0.16 %	0.06 % ± 0.12 %	0.01 % ± 0.02 %
M+5	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.09 % ± 0.16 %	0.01 % ± 0.02 %
M+6	0.01 % ± 0.02 %	0.00 % ± 0.00 %	0.11 % ± 0.18 %	0.00 % ± 0.01 %

3 mM [U-$^{13}C_3$]serine *Fth* LVS				
	Glucose in glycogen	Free fructose	Muramic acid	Glucosamine
M+1	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.84 % ± 0.88 %	0.82 % ± 0.90 %
M+2	0.00 % ± 0.00 %	0.23 % ± 0.44 %	0.72 % ± 0.50 %	0.41 % ± 0.44 %
M+3	0.00 % ± 0.00 %	0.74 % ± 0.49 %	0.06 % ± 0.14 %	0.04 % ± 0.09 %

M+4	0.01 % ± 0.00 %	0.12 % ± 0.20 %	0.04 % ± 0.10 %	0.01 % ± 0.01 %
M+5	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.07 % ± 0.14 %	0.01 % ± 0.02 %
M+6	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.09 % ± 0.29 %	0.01 % ± 0.02 %

3 mM [U-$^{13}C_3$]serine *Fno*				
	Glucose in glycogen	Free fructose	Muramic acid	Glucosamine
M+1	0.00 % ± 0.00 %	0.07 % ± 0.17 %	3.66 % ± 3.30 %	1.23 % ± 0.79 %
M+2	0.00 % ± 0.00 %	0.08 % ± 0.20 %	0.39 % ± 0.84 %	0.50 % ± 0.42 %
M+3	0.00 % ± 0.00 %	0.56 % ± 0.45 %	0.37 % ± 0.76 %	0.01 % ± 0.04 %
M+4	0.00 % ± 0.00 %	0.21 % ± 0.19 %	0.35 % ± 0.58 %	0.00 % ± 0.00 %
M+5	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.20 % ± 0.53 %	0.01 % ± 0.03 %
M+6	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.51 % ± 0.84 %	0.00 % ± 0.01 %

Supplemental Table S24 Relative fractions of isotopologues (mol%) of sugars from experiments with *Fth* WT, *Fth* LVS and *Fno* grown in medium T supplemented with 25 mM [U-$^{13}C_3$]glycerol. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

25 mM [U-$^{13}C_3$]glycerol *Fth* WT				
	Glucose in glycogen	Free fructose	Muramic acid	Glucosamine
M+1	0.00 % ± 0.00 %	0.05 % ± 0.08 %	2.46 % ± 2.50 %	0.89 % ± 0.67 %
M+2	0.02 % ± 0.04 %	0.00 % ± 0.00 %	0.81 % ± 1.14 %	0.38 % ± 0.56 %
M+3	1.12 % ± 0.12 %	0.89 % ± 0.65 %	6.26 % ± 2.14 %	1.76 % ± 0.43 %
M+4	0.01 % ± 0.02 %	0.52 % ± 0.44 %	1.20 % ± 1.05 %	0.03 % ± 0.05 %
M+5	0.01 % ± 0.01 %	0.00 % ± 0.00 %	0.13 % ± 0.35 %	0.11 % ± 0.07 %
M+6	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.26 % ± 0.38 %	0.07 % ± 0.07 %

25 mM [U-$^{13}C_3$]glycerol *Fth* LVS				
	Glucose in glycogen	Free fructose	Muramic acid	Glucosamine
M+1	0.04 % ± 0.07 %	0.00 % ± 0.00 %	1.06 % ± 1.43 %	0.63 % ± 0.63 %
M+2	0.00 % ± 0.00 %	0.01 % ± 0.03 %	0.93 % ± 0.72 %	0.50 % ± 0.48 %

M+3	0.79 % ± 0.07 %	1.09 % ± 0.47 %	4.37 % ± 1.17 %	1.19 % ± 0.24 %
M+4	0.01 % ± 0.01 %	0.06 % ± 0.10 %	0.77 % ± 0.67 %	0.02 % ± 0.04 %
M+5	0.01 % ± 0.01 %	0.00 % ± 0.00 %	0.02 % ± 0.07 %	0.02 % ± 0.03 %
M+6	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.29 % ± 0.64 %	0.06 % ± 0.13 %

25 mM [U-$^{13}C_3$]glycerol *Fno*				
	Glucose in glycogen	Free fructose	Muramic acid	Glucosamine
M+1	0.01 % ± 0.03 %	0.48 % ± 0.69 %	3.63 % ± 5.55 %	1.81 % ± 2.11 %
M+2	0.12 % ± 0.08 %	0.27 % ± 0.52 %	2.75 % ± 4.68 %	0.70 % ± 0.55 %
M+3	1.66 % ± 0.31 %	1.96 % ± 0.56 %	21.72 % ± 11.85 %	4.52 % ± 1.27 %
M+4	0.04 % ± 0.02 %	0.46 % ± 0.43 %	2.27 % ± 1.62 %	0.33 % ± 0.90 %
M+5	0.03 % ± 0.01 %	0.00 % ± 0.00 %	2.93 % ± 5.45 %	0.29 % ± 0.42 %
M+6	0.15 % ± 0.04 %	0.00 % ± 0.00 %	0.75 % ± 1.19 %	0.20 % ± 0.24 %

Supplemental Table S25 Composition of amino acids, polar metabolites and glucose in medium T. Mean and SD from two independent experiments are shown.

Amino acids in Medium T umol/mL	
Ala	5.01 ± 0.38
Gly	5.75 ± 0.33
Val	5.36 ± 0.80
Nor	1.00 ± 0.00
Leu	6.43 ± 1.28
Ile	3.45 ± 0.69
Pro	8.96 ± 1.30
Met	0.29 ± 0.20
Ser	3.06 ± 0.66
Thr	0.76 ± 0.19
Phe	1.90 ± 0.57
Asp	7.54 ± 1.32

Glu	14.64 ± 2.79
Lys	4.07 ± 1.15
His	0.49 ± 0.21
Tyr	0.91 ± 0.18

Polar metabolites in Medium T umol/mL	
Lactate	0.07 ± 0.00
Glycolic acid	0.01 ± 0.00
Ala	0.47 ± 0.01
Gly	0.19 ± 0.00
Val	0.47 ± 0.01
Leu	1.11 ± 0.02
Ile	0.31 ± 0.01
Pro	0.53 ± 0.02
5-oxo-Pro	0.74 ± 0.02
Met	0.18 ± 0.01
Ser	0.53 ± 0.04
Thr	0.25 ± 0.03
Phe	0.40 ± 0.01
Asp	0.70 ± 0.01
Glu	1.04 ± 0.02
Asn	0.26 ± 0.00
Lys	0.49 ± 0.01
Citrate	0.89 ± 0.02
Tyr	0.13 ± 0.00

Glucose in Medium T umol/mL	
Glucose	75.31 ± 9.42

Supplemental Table S26 Composition of medium T in the [U-$^{13}C_6$]glucose, [1,2-$^{13}C_2$]glucose, [U-$^{13}C_3$]serine, or [U-$^{13}C_3$]glycerol labelled experiments.

[U-$^{13}C_6$] Glucose Medium T	
brain heart infusion broth (Fa. Difco)	10 g/L
bacto trypton (Fa. Difco)	10 g/L
technical casamino acid (Fa. Difco)	10 g/L
$MgSO_4$	0.005 g/L
$FeSO_4$	0.1 g/L
NaCitrat	1.2 g/L
KCl	0.2 g/L
K_2HPO_4	0.4 g/L
L-Cystein	0.6 g/L
Glucose	15 g/L
U-$^{13}C_6$-Glucose	2 g/L

[1,2-$^{13}C_2$] Glucose Medium T	
brain heart infusion broth (Fa. Difco)	10 g/L
bacto trypton (Fa. Difco)	10 g/L
technical casamino acid (Fa. Difco)	10 g/L
$MgSO_4$	0.005 g/L
$FeSO_4$	0.1 g/L
NaCitrat	1.2 g/L
KCl	0.2 g/L
K_2HPO_4	0.4 g/L
L-Cystein	0.6 g/L
Glucose	15 g/L
1,2-$^{13}C_2$-Glucose	2 g/L

[U-$^{13}C_3$]serine Medium T

brain heart infusion broth (Fa. Difco)	10 g/L
bacto trypton (Fa. Difco)	10 g/L
technical casamino acid (Fa. Difco)	10 g/L
$MgSO_4$	0.005 g/L
$FeSO_4$	0.1 g/L
NaCitrat	1.2 g/L
KCl	0.2 g/L
K_2HPO_4	0.4 g/L
L-Cystein	0.6 g/L
Glucose	15 g/L
U-$^{13}C_3$-serine	0.3 g/L

[U-$^{13}C_3$]glycerol Medium T	
brain heart infusion broth (Fa. Difco)	10 g/L
bacto trypton (Fa. Difco)	10 g/L
technical casamino acid (Fa. Difco)	10 g/L
$MgSO_4$	0.005 g/L
$FeSO_4$	0.1 g/L
NaCitrat	1.2 g/L
KCl	0.2 g/L
K_2HPO_4	0.4 g/L
L-cysteine	0.6 g/L
glucose	15 g/L
U-$^{13}C_3$-glycerol	2.5 g/L

Supplemental Table S27 Retention time and mass fragments of derivatized metabolites used for isotopologue calculations.

Metabolite	Retention time [min]	[M-57]+	[M-85]+
Ala	6.6	m/z 260	

Gly	6.9	m/z 246	
Val	8.4	m/z 288	
Leu	9.0		m/z 274
Ile	9.5		m/z 274
Pro	10.1		m/z 285
Met	13.0	m/z 320	
Ser	13.3	m/z 390	
Thr	13.7	m/z 404	
Phe	14.7	m/z 336	
Asp	15.5	m/z 418	
Glu	17.0	m/z 432	
Lys	18.3	m/z 431	
His	20.2	m/z 440	
Tyr	21.2	m/z 466	

Metabolite	Retention time [min]	[M-57]+	[M-85]+
Lactate	17.6	m/z 261	
Glycolic acid	18.2	m/z 247	
Alanine	19.3	m/z 260	
Glycine	20.2	m/z 246	
Oxalic aicd	20.9	m/z 261	
3-hydroxy butyric acid	21.4	m/z 275	
Valine	23.6	m/z 288	
Leucine	25.2		m/z 274
Isoleucine	26.3		m/z 274
Succinate	27.3	m/z 289	
Fumarate	28.4	m/z 287	
Glycerol	31.5	m/z 377	

Methionine	34.2	m/z 320	
Serine	35.0	m/z 390	
Threonine	35.9	m/z 404	
Phenyl alanine	37.9	m/z 336	
Malate	38.4	m/z 419	
Aspartate	40.1	m/z 418	
Glutamate	43.5	m/z 432	
Palmitate	43.9	m/z 313	
Oleic acid	48.8	m/z 339	
Stearate	49.5	m/z 341	
Citrate	53.5	m/z 591	

Metabolite	Retention time [min]	[M-15]+	[M-176]+
Gla-1	31.7	m/z 452	
Gla-2	32.8	m/z 452	
Mur-1	35.9		m/z 434
Mur-2	36.4		m/z 434

Metabolite	Retention time [min]	[M-15]+
Glucose in glycogen	8.0	m/z 287

Metabolite	Retention time [min]	[M-15]+
Fructose	33.8	m/z 554

Supplemental Tables S28 ^{13}C-Excess (mol%) of protein-bound amino acids from [U-$^{13}C_6$]glucose

^{13}C-Excess (mol%) of protein-bound amino acids from experiments with *F.* W12-1067 WT and Δmyo-inositol mutant grown in medium T supplemented with 11 mM [U-$^{13}C_6$]glucose. Mean and SD from two or three independent experiments are shown.

	WT	Δmyo-inositol mutant
Ala	6.48% ± 0.21%	9.39% ± 0.67%

Asp	0.61%	±	0.13%	0.89%	±	0.18%
Glu	2.16%	±	0.04%	2.31%	±	0.21%
Gly	0.30%	±	0.05%	0.36%	±	0.13%
His	0.21%	±	0.17%	0.20%	±	0.18%
Ile	0.10%	±	0.02%	0.11%	±	0.05%
Leu	0.00%	±	0.00%	0.04%	±	0.03%
Lys	0.05%	±	0.02%	0.08%	±	0.04%
Met	0.30%	±	0.20%	0.24%	±	0.45%
Phe	0.25%	±	0.04%	0.44%	±	0.15%
Pro	0.18%	±	0.03%	0.36%	±	0.13%
Ser	1.85%	±	0.10%	2.70%	±	0.17%
Thr	0.58%	±	0.06%	0.58%	±	0.14%
Tyr	1.73%	±	0.06%	1.51%	±	0.16%
Val	0.18%	±	0.05%	0.07%	±	0.05%

Supplemental Tables S29 ^{13}C-Excess (mol%) of protein-bound amino acids from [1,2-$^{13}C_2$]glucose

^{13}C-Excess (mol%) of protein-bound amino acids from experiments with *F.* W12-1067 WT grown in medium T supplemented with 11 mM [1,2-$^{13}C_2$]glucose. Mean and SD from two independent experiments are shown.

	WT			Δmyo-inositol mutant
Ala	2.61%	±	0.11%	
Asp	0.30%	±	0.12%	
Glu	1.37%	±	0.10%	
Gly	0.30%	±	0.13%	
His	0.53%	±	0.49%	
Ile	0.10%	±	0.04%	
Leu	0.00%	±	0.00%	
Lys	0.08%	±	0.03%	
Met	0.83%	±	0.73%	
Phe	0.17%	±	0.04%	
Pro	0.19%	±	0.06%	
Ser	0.70%	±	0.07%	
Thr	0.72%	±	0.14%	
Tyr	0.70%	±	0.03%	

Val	0.17%	±	0.07%		

Supplemental Tables S30 ^{13}C-Excess (mol%) of protein-bound amino acids from [U-$^{13}C_3$]serine

^{13}C-Excess (mol%) of protein-bound amino acids from experiments with *F.* W12-1067 WT grown in medium T supplemented with 3 mM [U-$^{13}C_3$]serine. Mean and SD from two independent experiments are shown.

	WT			Δmyo-inositol mutant
Ala	3.92%	±	0.29%	
Asp	0.26%	±	0.07%	
Glu	1.20%	±	0.04%	
Gly	3.20%	±	0.44%	
His	0.06%	±	0.04%	
Ile	0.09%	±	0.03%	
Leu	0.01%	±	0.01%	
Lys	0.10%	±	0.03%	
Met	0.24%	±	0.43%	
Phe	0.07%	±	0.02%	
Pro	0.22%	±	0.06%	
Ser	14.79%	±	1.60%	
Thr	0.51%	±	0.07%	
Tyr	0.13%	±	0.03%	
Val	0.01%	±	0.01%	

Supplemental Tables S31 ^{13}C-Excess (mol%) of protein-bound amino acids from [U-$^{13}C_3$] glycerol

^{13}C-Excess (mol%) of protein-bound amino acids from experiments with *F.* W12-1067 WT and Δmyo-inositol mutant grown in medium T supplemented with 25 mM [U-$^{13}C_3$] glycerol. Mean and SD from two independent experiments are shown.

	WT			Δmyo-inositol mutant		
Ala	13.70%	±	1.08%	15.49%	±	1.61%
Asp	1.54%	±	0.15%	0.90%	±	0.12%
Glu	4.72%	±	0.31%	3.99%	±	0.14%
Gly	0.50%	±	0.20%	0.39%	±	0.16%

His	0.07%	±	0.03%	0.15%	±	0.11%
Ile	0.11%	±	0.01%	0.12%	±	0.03%
Leu	0.09%	±	0.03%	0.03%	±	0.03%
Lys	0.14%	±	0.02%	0.06%	±	0.05%
Met	0.15%	±	0.17%	0.08%	±	0.06%
Phe	0.29%	±	0.12%	0.55%	±	0.10%
Pro	0.27%	±	0.03%	0.33%	±	0.07%
Ser	4.30%	±	0.43%	3.90%	±	0.38%
Thr	0.45%	±	0.08%	0.66%	±	0.13%
Tyr	2.77%	±	0.07%	2.22%	±	0.19%
Val	0.04%	±	0.03%	0.06%	±	0.06%

Supplemental Table S32 ^{13}C-Excess (mol%) of polar metabolites from [U-$^{13}C_6$]glucose

^{13}C-Excess (mol%) of polar metabolites from experiments with *F.* W12-1067 WT and Δmyo-inositol mutant grown in medium T supplemented with 11 mM [U-$^{13}C_6$]glucose. Mean and SD from two independent experiments are shown.

	WT			Δmyo-inositol mutant		
Lactate	7.94%	±	0.96%	10.15%	±	0.61%
Glycolic acid	10.61%	±	0.89%	11.09%	±	0.77%
Alanine	9.82%	±	0.37%	11.18%	±	0.74%
Glycine	0.29%	±	0.06%	0.21%	±	0.05%
3-hydroxy butyric acid	8.19%	±	0.89%	7.26%	±	0.44%
Valine	0.18%	±	0.02%	0.01%	±	0.01%
leucine	0.02%	±	0.01%	0.07%	±	0.04%
Isoleucine	0.03%	±	0.04%	0.30%	±	0.07%
Succinic acid	2.34%	±	0.82%	1.73%	±	0.13%
Fumaric acid	0.93%	±	0.98%	1.00%	±	0.12%
Glycerol	5.74%	±	1.37%	6.65%	±	0.14%
Methionine	0.10%	±	0.03%	0.03%	±	0.03%
Serine	0.58%	±	0.16%	0.37%	±	0.14%

Threonine	0.21%	±	0.04%	0.17%	±	0.20%
Phenylalanine	0.59%	±	0.52%	0.33%	±	0.04%
Malic acid	5.89%	±	4.24%	1.79%	±	0.65%
Aspartate	0.28%	±	0.10%	0.46%	±	0.14%
Glutamate	4.10%	±	1.34%	2.31%	±	0.25%
Palmitic acid	6.18%	±	0.80%	5.57%	±	0.33%
Oleic acid	6.56%	±	1.15%	5.77%	±	0.42%
Stearic acid	7.85%	±	1.82%	5.83%	±	0.25%
Citric acid	0.73%	±	1.05%	1.02%	±	0.56%

Supplemental Table S33 ^{13}C-Excess (mol%) of polar metabolites from [1,2-$^{13}C_2$]glucose

^{13}C-Excess (mol%) of polar metabolites from experiments with *F.* W12-1067 WT grown in medium T supplemented with 11 mM [1,2-$^{13}C_2$]glucose. Mean and SD from two independent experiments are shown.

	WT			Δmyo-inositol mutant
Lactate	3.83%	±	0.07%	
Glycolic acid	5.69%	±	0.39%	
Alanine	3.69%	±	0.07%	
Glycine	0.23%	±	0.07%	
3-hydroxy butyric acid	4.28%	±	0.18%	
Valine	0.17%	±	0.05%	
leucine	0.03%	±	0.01%	
Isoleucine	0.02%	±	0.02%	
Succinic acid	0.79%	±	0.19%	
Fumaric acid	0.38%	±	0.24%	
Glycerol	2.51%	±	0.73%	
Methionine	0.11%	±	0.05%	
Serine	0.56%	±	0.12%	
Threonine	0.19%	±	0.06%	
Phenylalanine	0.11%	±	0.02%	

Malic acid	2.88%	± 1.06%	
Aspartate	0.16%	± 0.07%	
Glutamate	1.85%	± 0.09%	
Palmitic acid	3.18%	± 0.27%	
Oleic acid	3.57%	± 0.25%	
Stearic acid	3.78%	± 0.25%	
Citric acid	1.98%	± 1.26%	

Supplemental Table S34 ^{13}C-Excess (mol%) of polar metabolites from [U-$^{13}C_3$]serine

^{13}C-Excess (mol%) of polar metabolites from experiments with *F.* W12-1067 WT grown in medium T supplemented with 3 mM [U-$^{13}C_3$]serine. Mean and SD from three independent experiments are shown

	WT		Δmyo-inositol mutant
Lactate	0.59%	± 0.20%	
Glycolic acid	0.24%	± 0.16%	
Alanine	1.92%	± 0.26%	
Glycine	2.23%	± 0.23%	
3-hydroxy butyric acid	2.95%	± 0.12%	
Valine	0.08%	± 0.04%	
leucine	0.05%	± 0.02%	
Isoleucine	0.13%	± 0.08%	
Succinic acid	0.24%	± 0.04%	
Fumaric acid	0.24%	± 0.23%	
Glycerol	0.53%	± 0.42%	
Methionine	0.16%	± 0.08%	
Serine	4.02%	± 0.70%	
Threonine	0.24%	± 0.21%	
Phenylalanine	0.19%	± 0.04%	
Malic acid	4.08%	± 2.45%	
Aspartate	0.09%	± 0.10%	

Glutamate	0.64%	±	0.09%		
Palmitic acid	2.84%	±	0.16%		
Oleic acid	5.69%	±	0.32%		
Stearic acid	3.55%	±	0.53%		
Citric acid	0.29%	±	0.47%		

Supplemental Table S35 ^{13}C-Excess (mol%) of polar metabolites from [U-$^{13}C_3$] glycerol

^{13}C-Excess (mol%) of polar metabolites from experiments with *F.* W12-1067 WT and Δmyo-inositol mutant grown in medium T supplemented with 25 mM [U-$^{13}C_3$] glycerol. Mean and SD from two independent experiments are shown.

	WT			Δmyo-inositol mutant		
Lactate	11.33%	±	0.96%	13.06%	±	0.51%
Glycolic acid	6.33%	±	3.33%	6.72%	±	0.98%
Alanine	18.31%	±	1.94%	19.79%	±	1.71%
Glycine	0.77%	±	0.46%	0.43%	±	0.16%
3-hydroxy butyric acid	15.96%	±	1.80%	14.95%	±	0.27%
Valine	0.04%	±	0.04%	0.01%	±	0.01%
leucine	0.01%	±	0.01%	0.05%	±	0.04%
Isoleucine	0.03%	±	0.02%	0.31%	±	0.06%
Succinic acid	4.20%	±	0.34%	5.48%	±	0.24%
Fumaric acid	0.65%	±	0.62%	0.89%	±	0.28%
Glycerol	95.65%	±	0.32%	95.09%	±	0.06%
Methionine	0.04%	±	0.05%	0.05%	±	0.05%
Serine	0.69%	±	0.10%	0.88%	±	0.17%
Threonine	0.23%	±	0.12%	0.08%	±	0.02%
Phenylalanine	0.36%	±	0.12%	0.45%	±	0.03%
Malic acid	18.71%	±	7.77%	6.27%	±	2.85%
Aspartate	0.18%	±	0.13%	0.23%	±	0.08%
Glutamate	5.68%	±	0.39%	6.06%	±	0.22%
Palmitic acid	4.47%	±	1.11%	10.63%	±	0.73%

Oleic acid	10.69%	±	2.20%	9.55%	±	1.60%
Stearic acid	5.32%	±	1.33%	11.27%	±	1.14%
Citric acid	0.45%	±	0.31%	1.38%	±	0.95%

Supplemental Table S36 ^{13}C-Excess (mol%) of sugars from [U-$^{13}C_6$]glucose

^{13}C-Excess (mol%) of sugars from experiments with *F.* W12-1067 WT and Δmyo-inositol mutant grown in medium T supplemented with 11 mM [U-$^{13}C_6$]glucose. Mean and SD from three independent experiments are shown.

	WT			Δmyo-inositol mutant		
Glucose in glycogen	9.55%	±	0.87%	11.26 %	±	1.36 %
Free fructose	12.50%	±	0.43 %	13.54 %	±	0.95 %
Glucosamine	10.15 %	±	1.30%	11.27 %	±	3.38 %
Muramic acid	12.17 %	±	2.99 %			

Supplemental Table S37 ^{13}C-Excess (mol%) of sugars from [1,2-$^{13}C_2$]glucose

^{13}C-Excess (mol%) of sugars from experiments with *F.* W12-1067 WT grown in medium T supplemented with 11 mM [1,2-$^{13}C_2$]glucose. Mean and SD from two independent experiments are shown.

	WT			Δmyo-inositol mutant
Glucose in glycogen	4.18%	±	0.09%	
Free fructose	4.71%	±	0.33%	
Glucosamine	5.28 %	±	0.23 %	
Muramic acid	8.19 %	±	0.55 %	

Supplemental Table S38 ^{13}C-Excess (mol%) of sugars from [U-$^{13}C_3$]serine

^{13}C-Excess (mol%) of sugars from experiments with *F.* W12-1067 WT grown in medium T supplemented with 3 mM [U-$^{13}C_3$]serine. Mean and SD from three independent experiments are shown.

	WT			Δmyo-inositol mutant
Glucose in glycogen	0.10%	±	0.15%	
Free fructose	0.55%	±	0.08%	
Glucosamine	0.15 %	±	0.09 %	

Muramic acid	1.23 %	±	1.04 %		

Supplemental Table S39 ^{13}C-Excess (mol%) of sugars from [U-$^{13}C_3$] glycerol

^{13}C-Excess (mol%) of sugars from experiments with *F.* W12-1067 WT and Δmyo-inositol mutant grown in medium T supplemented with 25 mM [U-$^{13}C_3$] glycerol. Mean and SD from three independent experiments are shown.

	WT			Δmyo-inositol mutant		
Glucose in glycogen	0.63%	±	0.19%	0.61%	±	0.12%
Free fructose	0.56%	±	0.12%	0.58%	±	0.29%
Glucosamine	7.91%	±	1.85%	9.60 %	±	0.43 %
Muramic acid	14.31%	±	1.64%			

Supplemental Table S40 Relative fractions of isotopologues (mol%) of amino acids from experiments with *F.* W12-1067 WT and Δmyo-inositol mutant grown in medium T supplemented with 11 mM [U-$^{13}C_6$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

11 mM [U-$^{13}C_6$]glucose *F.* W12-1067 WT												
	Alanine			Glutamate			Serine			Tyrosine		
M+1	1.51 %	±	0.30 %	2.98 %	±	0.24 %	0.01 %	±	0.04 %	0.33 %	±	0.39 %
M+2	1.04 %	±	0.10 %	3.46 %	±	0.13 %	0.00 %	±	0.00 %	1.50 %	±	0.21 %
M+3	5.28 %	±	0.19 %	0.22 %	±	0.05 %	1.85 %	±	0.10 %	1.70 %	±	0.10 %
M+4				0.04 %	±	0.03 %				0.78 %	±	0.07 %
M+5				0.02 %	±	0.01 %				0.34 %	±	0.03 %
M+6										0.21 %	±	0.04 %
M+7										0.01 %	±	0.02 %
M+8										0.03 %	±	0.02 %
M+9										0.08 %	±	0.01 %

11 mM [U-$^{13}C_6$]glucose Δmyo-inositol mutant												
	Alanine			Glutamate			Serine			Tyrosine		
M+1	1.98 %	±	0.24 %	2.95 %	±	0.59 %	0.09 %	±	0.16 %	0.23 %	±	0.31 %
M+2	1.37 %	±	0.14 %	3.73 %	±	0.27 %	0.11 %	±	0.12 %	1.27 %	±	0.31 %

M+3	7.82 %	±	0.55 %	0.24 %	±	0.04 %	2.60 %	±	0.17 %	1.50 %	±	0.12 %
M+4				0.07 %	±	0.03 %				0.77 %	±	0.03 %
M+5				0.02 %	±	0.01 %				0.37 %	±	0.06 %
M+6										0.17 %	±	0.03 %
M+7										0.04 %	±	0.04 %
M+8										0.00 %	±	0.01 %
M+9										0.00 %	±	0.00 %

Supplemental Table S41 Relative fractions of isotopologues (mol%) of amino acids from experiments with *F.* W12-1067 WT grown in medium T supplemented with 11 mM [1,2-$^{13}C_2$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

11 mM [1,2-$^{13}C_2$]glucose *F.* W12-1067 WT												
	Alanine			Glutamate			Serine			Tyrosine		
M+1	0.28 %	±	0.32 %	1.99 %	±	0.47 %	0.03 %	±	0.06 %	0.00 %	±	0.00 %
M+2	3.70 %	±	0.16 %	2.32 %	±	0.25 %	0.63 %	±	0.07 %	2.48 %	±	0.33 %
M+3	0.05 %	±	0.03 %	0.05 %	±	0.04 %	0.27 %	±	0.04 %	0.21 %	±	0.12 %
M+4				0.00 %	±	0.01 %				0.02 %	±	0.02 %
M+5				0.01 %	±	0.00 %				0.06 %	±	0.05 %
M+6										0.01 %	±	0.02 %

M+7								0.00 %	±	0.00 %
M+8								0.02 %	±	0.02 %
M+9								0.01 %	±	0.02 %
	Ala-232									
M+1	0.01 %	±	0.02 %							
M+2	3.71 %	±	0.16 %							

Supplemental Table S42 Relative fractions of isotopologues (mol%) of amino acids from experiments with *F.* W12-1067 WT grown in medium T supplemented with 3 mM [U-$^{13}C_3$]serine. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

3 mM [U-$^{13}C_3$]serine *F.* W12-1067 WT											
	Alanine			Glutamate			Glycine			Serine		
M+1	0.64 %	±	0.20 %	1.64 %	±	0.25 %	0.59 %	±	0.12 %	1.37 %	±	0.62 %
M+2	0.36 %	±	0.04 %	2.15 %	±	0.18 %	2.91 %	±	0.39 %	1.04 %	±	0.25 %
M+3	3.47 %	±	0.25 %	0.01 %	±	0.03 %				13.63 %	±	1.24 %
M+4				0.00 %	±	0.00 %						
M+5				0.01 %	±	0.01 %						

Supplemental Table S43 Relative fractions of isotopologues (mol%) of amino acids from experiments with *F.* W12-1067 WT and Δmyo-inositol mutant grown in medium T supplemented with 25 mM [U-$^{13}C_3$]glycerol. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

25 mM [U-$^{13}C_3$]glycerol *F.* W12-1067 WT																
	Alanine			Aspartate			Glutamate			Serine			Tyrosine			
M+1	1.76 %	±	0.21 %	2.61 %	±	0.12 %	4.85 %	±	0.11 %	0.59 %	±	0.65 %	0.26 %	±	0.25 %	
M+2	1.12 %	±	0.14 %	1.29 %	±	0.20 %	7.21 %	±	0.46 %	0.00 %	±	0.00 %	2.93 %	±	0.24 %	
M+3	12.37 %	±	0.95 %	0.32 %	±	0.05 %	0.93 %	±	0.14 %	4.10 %	±	0.64 %	3.29 %	±	0.27 %	
M+4				0.00 %	±	0.00 %	0.32 %	±	0.06 %				0.07 %	±	0.05 %	
M+5							0.05 %	±	0.01 %				1.32 %	±	0.09 %	
M+6													0.30 %	±	0.04 %	
M+7													0.00 %	±	0.00 %	
M+8													0.03 %	±	0.03 %	
M+9													0.00 %	±	0.00 %	

25 mM [U-$^{13}C_3$]glycerol Δmyo-inositol mutant																
	Alanine			Aspartate			Glutamate			Serine			Tyrosine			
M+1	1.97 %	±	0.48 %	1.23 %	±	0.49 %	3.47 %	±	0.49 %	0.00 %	±	0.00 %	0.39 %	±	0.34 %	
M+2	1.29 %	±	0.14 %	0.80 %	±	0.25 %	6.40 %	±	0.28 %	0.04 %	±	0.07 %	1.99 %	±	0.32 %	
M+3	13.97 %	±	1.37 %	0.24 %	±	0.13 %	0.72 %	±	0.08 %	3.87 %	±	0.40 %	2.42 %	±	0.20 %	
M+4				0.00 %	±	0.00 %	0.32 %	±	0.03 %				0.02 %	±	0.03 %	

M+5			0.05 % ± 0.01 %		1.18 % ± 0.07 %
M+6					0.28 % ± 0.08 %
M+7					0.00 % ± 0.00 %
M+8					0.08 % ± 0.06 %
M+9					0.00 % ± 0.00 %

Supplemental Table S44 Relative fractions of isotopologues (mol%) of polar metabolites from experiments with *F.* W12-1067 WT and Δmyo-inositol mutant grown in medium T supplemented with 11 mM [U-$^{13}C_6$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

11 mM [U-$^{13}C_6$]glucose *F.* W12-1067 WT						
	Lactate	Glycolic acid	Alanine	3-Hydroxybutyric acid	Succinate	Fumarate
M+1	0.80 % ± 0.14 %	1.05 % ± 0.29 %	2.15 % ± 0.10 %	2.60 % ± 0.89 %	0.78 % ± 0.20 %	0.00 % ± 0.00 %
M+2	1.16 % ± 0.12 %	10.26 % ± 0.65 %	1.55 % ± 0.04 %	12.31 % ± 0.76 %	2.33 % ± 0.16 %	0.18 % ± 0.27 %
M+3	7.12 % ± 0.56 %		8.12 % ± 0.28 %	0.22 % ± 0.33 %	0.50 % ± 0.08 %	0.22 % ± 0.23 %
M+4				0.44 % ± 0.32 %	0.03 % ± 0.04 %	0.17 % ± 0.24 %

11 mM [U-$^{13}C_6$]glucose *F.* W12-1067 WT						
	Glycerol	Malate	Glutamate	Palmitate	Oelic acid	Stearic acid
M+1	2.01 % ± 0.31 %	2.80 % ± 4.51 %	4.29 % ± 0.35 %	2.86 % ± 0.20 %	5.42 % ± 0.21 %	5.06 % ± 0.22 %

M+2	0.88 %	±	0.29 %	5.47 %	±	6.00 %	5.05 %	±	0.17 %	21.52 %	±	0.69 %	24.74 %	±	0.38 %	24.19 %	±	0.64 %
M+3	3.32 %	±	0.35 %	0.66 %	±	1.42 %	0.25 %	±	0.08 %	1.91 %	±	0.09 %	2.65 %	±	0.10 %	3.21 %	±	0.13 %
M+4				2.74 %	±	3.27 %	0.12 %	±	0.02 %	6.44 %	±	0.19 %	6.93 %	±	0.28 %	8.56 %	±	0.27 %
M+5							0.03 %	±	0.01 %	0.53 %	±	0.05 %	0.79 %	±	0.04 %	1.07 %	±	0.02 %
M+6										1.25 %	±	0.05 %	1.43 %	±	0.09 %	2.10 %	±	0.12 %
M+7										0.06 %	±	0.02 %	0.16 %	±	0.05 %	0.23 %	±	0.02 %
M+8										0.17 %	±	0.02 %	0.23 %	±	0.02 %	0.41 %	±	0.05 %
M+9										0.01 %	±	0.01 %	0.03 %	±	0.01 %	0.05 %	±	0.01 %
M+10										0.03 %	±	0.02 %	0.04 %	±	0.02 %	0.09 %	±	0.02 %
M+11										0.00 %	±	0.00 %	0.00 %	±	0.01 %	0.01 %	±	0.01 %
M+12										0.00 %	±	0.00 %	0.01 %	±	0.01 %	0.00 %	±	0.00 %
M+13										0.06 %	±	0.02 %	0.00 %	±	0.01 %	0.00 %	±	0.00 %
M+14										0.01 %	±	0.02 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %
M+15										0.06 %	±	0.02 %	0.00 %	±	0.00 %	0.00 %	±	0.01 %
M+16										0.02 %	±	0.02 %	0.00 %	±	0.01 %	0.00 %	±	0.00 %
M+17													0.00 %	±	0.00 %	0.04 %	±	0.03 %
M+18													0.02 %	±	0.01 %	0.01 %	±	0.01 %

11 mM [U-$^{13}C_6$]glucose Δmyo-inositol mutant						
	Lactate	Glycolic acid	Alanine	3-Hydroxybutyric acid	Succinate	Fumarate
M+1	0.66 % ± 0.27 %	0.87 % ± 0.18 %	2.11 % ± 0.25 %	2.61 % ± 0.69 %	0.16 % ± 0.18 %	0.00 % ± 0.00 %
M+2	1.25 % ± 0.17 %	10.65 % ± 0.70 %	1.60 % ± 0.13 %	11.57 % ± 0.69 %	2.03 % ± 0.17 %	0.00 % ± 0.00 %
M+3	9.10 % ± 0.43 %		9.41 % ± 0.59 %	0.43 % ± 0.17 %	0.87 % ± 0.03 %	0.51 % ± 0.16 %
M+4				0.51 % ± 0.27 %	0.02 % ± 0.02 %	0.62 % ± 0.19 %

11 mM [U-$^{13}C_6$]glucose Δmyo-inositol mutant						
	Glycerol	Malate	Glutamate	Palmitate	Oelic acid	Stearic acid
M+1	1.11 % ± 0.37 %	2.12 % ± 1.31 %	2.55 % ± 0.67 %	2.88 % ± 0.33 %	5.18 % ± 0.67 %	4.77 % ± 0.17 %
M+2	1.27 % ± 0.17 %	2.05 % ± 2.00 %	3.79 % ± 0.32 %	20.49 % ± 0.66 %	23.21 % ± 1.22 %	20.57 % ± 0.86 %
M+3	5.43 % ± 0.19 %	0.00 % ± 0.00 %	0.39 % ± 0.07 %	2.18 % ± 0.18 %	2.78 % ± 0.30 %	2.96 % ± 0.17 %
M+4		0.24 % ± 0.38 %	0.04 % ± 0.03 %	6.23 % ± 0.49 %	6.96 % ± 0.57 %	7.09 % ± 0.37 %
M+5			0.02 % ± 0.01 %	0.61 % ± 0.07 %	0.81 % ± 0.11 %	0.95 % ± 0.10 %
M+6				1.22 % ± 0.14 %	1.46 % ± 0.17 %	1.62 % ± 0.14 %
M+7				0.10 % ± 0.03 %	0.13 % ± 0.03 %	0.19 % ± 0.03 %
M+8				0.14 % ± 0.03 %	0.23 % ± 0.04 %	0.28 % ± 0.03 %
M+9				0.01 % ± 0.00 %	0.02 % ± 0.01 %	0.03 % ± 0.01 %

M+10				0.00 % ± 0.00 %	0.01 % ± 0.01 %	0.04 % ± 0.01 %
M+11				0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+12				0.01 % ± 0.01 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+13				0.02 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+14				0.01 % ± 0.01 %	0.01 % ± 0.00 %	0.00 % ± 0.00 %
M+15				0.06 % ± 0.00 %	0.01 % ± 0.00 %	0.00 % ± 0.00 %
M+16				0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+17					0.00 % ± 0.00 %	0.13 % ± 0.05 %
M+18					0.00 % ± 0.00 %	0.04 % ± 0.02 %

Supplemental Table S45 Relative fractions of isotopologues (mol%) of polar metabolites from experiments with *F.* W12-1067 WT grown in medium T supplemented with 11 mM [1,2-$^{13}C_2$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from two independent experiments are shown.

11 mM [1,2-13C2]glucose *F.* W12-1067 WT						
	Lactate	Glycolic acid	Alanine	3-Hydroxybutyric acid	Succinate	Fumarate
M+1	0.08 % ± 0.10 %	0.67 % ± 0.35 %	0.82 % ± 0.09 %	1.58 % ± 0.46 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+2	5.50 % ± 0.13 %	5.35 % ± 0.26 %	5.02 % ± 0.12 %	7.24 % ± 0.35 %	1.03 % ± 0.38 %	0.10 % ± 0.18 %
M+3	0.14 % ± 0.02 %		0.07 % ± 0.04 %	0.17 % ± 0.22 %	0.36 % ± 0.04 %	0.30 % ± 0.35 %
M+4				0.14 % ± 0.15 %	0.01 % ± 0.01 %	0.10 % ± 0.14 %

11 mM [1,2-13C2]glucose *F.* W12-1067 WT						
	Glycerol	Malate	Glutamate	Palmitate	Oelic acid	Stearic acid
M+1	1.39 % ± 0.73 %	1.80 % ± 1.70 %	2.94 % ± 0.56 %	0.87 % ± 0.28 %	3.25 % ± 0.07 %	3.54 % ± 0.15 %
M+2	2.91 % ± 0.91 %	1.84 % ± 1.41 %	3.06 % ± 0.31 %	16.21 % ± 0.63 %	19.56 % ± 0.78 %	19.70 % ± 0.46 %
M+3	0.11 % ± 0.12 %	1.62 % ± 1.80 %	0.01 % ± 0.01 %	0.50 % ± 0.11 %	1.10 % ± 0.20 %	1.25 % ± 0.18 %
M+4		0.29 % ± 0.72 %	0.01 % ± 0.01 %	2.81 % ± 0.30 %	3.24 % ± 0.23 %	3.85 % ± 0.35 %
M+5			0.02 % ± 0.01 %	0.08 % ± 0.05 %	0.16 % ± 0.04 %	0.25 % ± 0.08 %
M+6				0.34 % ± 0.11 %	0.41 % ± 0.09 %	0.56 % ± 0.13 %
M+7				0.02 % ± 0.02 %	0.04 % ± 0.03 %	0.04 % ± 0.02 %
M+8				0.03 % ± 0.03 %	0.04 % ± 0.03 %	0.09 % ± 0.04 %
M+9				0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.01 % ± 0.01 %
M+10				0.01 % ± 0.01 %	0.04 % ± 0.03 %	0.02 % ± 0.02 %
M+11				0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+12				0.00 % ± 0.01 %	0.01 % ± 0.02 %	0.00 % ± 0.00 %
M+13				0.04 % ± 0.02 %	0.01 % ± 0.01 %	0.00 % ± 0.00 %
M+14				0.01 % ± 0.01 %	0.02 % ± 0.01 %	0.00 % ± 0.00 %
M+15				0.07 % ± 0.04 %	0.01 % ± 0.01 %	0.00 % ± 0.00 %
M+16				0.00 % ± 0.00 %	0.01 % ± 0.01 %	0.00 % ± 0.00 %

M+17					0.01 % ± 0.01 %	0.00 % ± 0.00 %
M+18					0.02 % ± 0.02 %	0.00 % ± 0.00 %

Supplemental Table S46 Relative fractions of isotopologues (mol%) of polar metabolites from experiments with *F.* W12-1067 WT grown in medium T supplemented with 3 mM [U-$^{13}C_3$]serine. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

3 mM [U-$^{13}C_3$]serine *F.* W12-1067 WT							
	Alanine	Glycine	3-Hydroxybutyric acid	Serine	Palmitate	Oelic acid	Stearic acid
M+1	0.49 % ± 0.04 %	0.49 % ± 0.07 %	1.78 % ± 0.73 %	0.00 % ± 0.00 %	2.24 % ± 0.47 %	6.27 % ± 0.27 %	4.94 % ± 0.31 %
M+2	0.24 % ± 0.03 %	1.98 % ± 0.21 %	4.49 % ± 0.46 %	0.42 % ± 0.25 %	12.63 % ± 1.01 %	21.65 % ± 0.48 %	14.39 % ± 0.83 %
M+3	1.60 % ± 0.25 %		0.08 % ± 0.10 %	3.74 % ± 0.59 %	1.06 % ± 0.06 %	3.08 % ± 0.21 %	1.72 % ± 0.10 %
M+4			0.20 % ± 0.19 %		2.25 % ± 0.19 %	5.90 % ± 0.26 %	2.87 % ± 0.14 %
M+5					0.21 % ± 0.03 %	1.00 % ± 0.07 %	0.39 % ± 0.02 %
M+6					0.33 % ± 0.04 %	1.39 % ± 0.19 %	0.46 % ± 0.04 %
M+7					0.03 % ± 0.02 %	0.25 % ± 0.06 %	0.04 % ± 0.02 %
M+8					0.00 % ± 0.01 %	0.28 % ± 0.08 %	0.06 % ± 0.02 %
M+9					0.01 % ± 0.02 %	0.05 % ± 0.02 %	0.00 % ± 0.00 %
M+10					0.00 % ± 0.00 %	0.05 % ± 0.06 %	0.02 % ± 0.02 %
M+11					0.01 % ± 0.02 %	0.01 % ± 0.02 %	0.00 % ± 0.00 %

M+12					0.02 % ± 0.03 %	0.01 % ± 0.01 %	0.13 % ± 0.15 %
M+13					0.11 % ± 0.04 %	0.01 % ± 0.01 %	0.05 % ± 0.06 %
M+14					0.00 % ± 0.00 %	0.01 % ± 0.01 %	0.24 % ± 0.26 %
M+15					0.01 % ± 0.01 %	0.01 % ± 0.02 %	0.07 % ± 0.08 %
M+16					0.03 % ± 0.04 %	0.05 % ± 0.06 %	0.04 % ± 0.05 %
M+17						0.01 % ± 0.02 %	0.01 % ± 0.01 %
M+18						0.00 % ± 0.00 %	0.02 % ± 0.04 %

Supplemental Table S47 Relative fractions of isotopologues (mol%) of polar metabolites from experiments with *F.* W12-1067 WT and Δmyo-inositol mutant grown in medium T supplemented with 25 mM [U-$^{13}C_3$]glycerol. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

25 mM [U-$^{13}C_3$]glycerol *F.* W12-1067 WT						
	Lactate	Glycolic acid	Alanine	3-Hydroxybutyric acid	Glycerol	Malate
M+1	0.09 % ± 0.15 %	0.37 % ± 0.45 %	1.74 % ± 0.50 %	4.41 % ± 1.04 %	0.05 % ± 0.03 %	16.16 % ± 9.69 %
M+2	0.56 % ± 0.16 %	6.14 % ± 3.24 %	1.46 % ± 0.28 %	21.77 % ± 1.12 %	2.42 % ± 0.04 %	6.81 % ± 8.27 %
M+3	10.93 % ± 0.83 %		16.76 % ± 1.61 %	0.93 % ± 1.40 %	94.03 % ± 0.32 %	3.01 % ± 5.18 %
M+4				3.28 % ± 1.88 %		9.01 % ± 8.00 %
M+5						
M+6						

M+7						
M+8						
M+9						

25 mM [U-$^{13}C_3$]glycerol *F.* W12-1067 WT				
	Glutamate	Palmitate	Oelic acid	Stearic acid
M+1	5.27 % ± 0.34 %	0.13 % ± 0.21 %	4.95 % ± 0.71 %	1.95 % ± 0.56 %
M+2	8.28 % ± 0.70 %	10.84 % ± 1.26 %	26.30 % ± 1.63 %	10.91 % ± 0.97 %
M+3	1.24 % ± 0.07 %	1.61 % ± 0.47 %	4.63 % ± 1.17 %	2.25 % ± 0.58 %
M+4	0.61 % ± 0.10 %	5.49 % ± 1.30 %	13.20 % ± 2.72 %	6.47 % ± 1.27 %
M+5	0.08 % ± 0.03 %	0.85 % ± 0.31 %	2.61 % ± 0.88 %	1.35 % ± 0.52 %
M+6		1.97 % ± 0.62 %	5.03 % ± 1.37 %	2.73 % ± 0.77 %
M+7		0.27 % ± 0.12 %	0.94 % ± 0.38 %	0.50 % ± 0.20 %
M+8		0.47 % ± 0.20 %	1.46 % ± 0.53 %	0.87 % ± 0.31 %
M+9		0.06 % ± 0.04 %	0.24 % ± 0.11 %	0.15 % ± 0.07 %
M+10		0.07 % ± 0.05 %	0.34 % ± 0.15 %	0.19 % ± 0.08 %
M+11		0.00 % ± 0.00 %	0.04 % ± 0.03 %	0.02 % ± 0.01 %
M+12		0.00 % ± 0.00 %	0.04 % ± 0.02 %	0.02 % ± 0.01 %

M+13		0.00 % ± 0.00 %	0.01 % ± 0.00 %	0.00 % ± 0.01 %
M+14		0.00 % ± 0.00 %	0.01 % ± 0.01 %	0.00 % ± 0.00 %
M+15		0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+16		0.00 % ± 0.00 %	0.00 % ± 0.01 %	0.00 % ± 0.00 %
M+17			0.00 % ± 0.00 %	0.08 % ± 0.09 %
M+18			0.00 % ± 0.00 %	0.03 % ± 0.04 %

25 mM [U-$^{13}C_3$]glycerol Δmyo-inositol mutant						
	Lactate	Glycolic acid	Alanine	3-Hydroxybutyric acid	Glycerol	Malate
M+1	0.10 % ± 0.13 %	0.14 % ± 0.09 %	2.25 % ± 0.33 %	4.19 % ± 0.49 %	0.03 % ± 0.02 %	5.39 % ± 2.19 %
M+2	0.81 % ± 0.10 %	6.65 % ± 0.98 %	1.75 % ± 0.29 %	22.60 % ± 0.67 %	2.44 % ± 0.05 %	5.01 % ± 4.77 %
M+3	12.49 % ± 0.43 %		17.87 % ± 1.41 %	0.49 % ± 0.19 %	93.45 % ± 0.07 %	2.55 % ± 2.31 %
M+4				2.24 % ± 0.20 %		0.50 % ± 0.70 %
M+5						
M+6						
M+7						
M+8						
M+9						

25 mM [U-$^{13}C_3$]glycerol Δmyo-inositol mutant				
	Glutamate	Palmitate	Oelic acid	Stearic acid
M+1	5.17 % ± 0.41 %	3.46 % ± 0.27 %	4.49 % ± 1.00 %	4.41 % ± 0.21 %
M+2	8.94 % ± 0.38 %	21.58 % ± 1.53 %	22.09 % ± 2.68 %	20.64 % ± 1.79 %
M+3	1.41 % ± 0.07 %	4.29 % ± 0.26 %	4.51 % ± 0.77 %	5.05 % ± 0.58 %
M+4	0.64 % ± 0.06 %	12.64 % ± 0.91 %	11.76 % ± 2.00 %	13.45 % ± 1.59 %
M+5	0.09 % ± 0.01 %	2.22 % ± 0.19 %	2.50 % ± 0.44 %	3.19 % ± 0.34 %
M+6		4.73 % ± 0.36 %	4.59 % ± 0.94 %	6.20 % ± 0.72 %
M+7		0.73 % ± 0.07 %	0.87 % ± 0.20 %	1.33 % ± 0.15 %
M+8		1.23 % ± 0.12 %	1.31 % ± 0.33 %	1.96 % ± 0.19 %
M+9		0.16 % ± 0.03 %	0.23 % ± 0.07 %	0.38 % ± 0.05 %
M+10		0.21 % ± 0.03 %	0.26 % ± 0.08 %	0.47 % ± 0.06 %
M+11		0.01 % ± 0.01 %	0.03 % ± 0.02 %	0.09 % ± 0.02 %
M+12		0.03 % ± 0.01 %	0.04 % ± 0.02 %	0.01 % ± 0.02 %
M+13		0.02 % ± 0.01 %	0.01 % ± 0.01 %	0.01 % ± 0.01 %
M+14		0.03 % ± 0.00 %	0.01 % ± 0.00 %	0.00 % ± 0.00 %
M+15		0.06 % ± 0.02 %	0.01 % ± 0.00 %	0.01 % ± 0.01 %
M+16		0.01 % ± 0.01 %	0.00 % ± 0.00 %	0.01 % ± 0.01 %

M+17			0.00 % ± 0.00 %	0.02 % ± 0.01 %	
M+18			0.00 % ± 0.00 %	0.01 % ± 0.00 %	

Supplemental Table S48 Relative fractions of isotopologues (mol%) of sugars from experiments with *F.* W12-1067 WT and Δmyo-inositol mutant grown in medium T supplemented with 11 mM [U-$^{13}C_6$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

11 mM [U-$^{13}C_6$]glucose *F.* W12-1067 WT				
	Glucose in glycogen	Free fructose	Muramic acid	Glucosamine
M+1	0.09 % ± 0.14 %	0.07 % ± 0.20 %	5.22 % ± 3.34 %	3.39 % ± 0.68 %
M+2	0.54 % ± 0.53 %	0.45 % ± 0.49 %	5.16 % ± 2.92 %	3.42 % ± 0.44 %
M+3	0.45 % ± 0.04 %	0.89 % ± 0.34 %	6.53 % ± 3.73 %	4.51 % ± 1.09 %
M+4	0.10 % ± 0.03 %	0.23 % ± 0.17 %	3.24 % ± 1.39 %	0.94 % ± 0.15 %
M+5	0.54 % ± 0.05 %	0.01 % ± 0.02 %	0.25 % ± 0.54 %	1.19 % ± 0.18 %
M+6	8.62 % ± 0.90 %	11.73 % ± 0.48 %	3.94 % ± 1.78 %	4.57 % ± 0.70 %

11 mM [U-$^{13}C_6$]glucose Δmyo-inositol mutant				
	Glucose in glycogen	Free fructose	Muramic acid	Glucosamine
M+1	0.09 % ± 0.10 %	0.00 % ± 0.00 %		2.39 % ± 1.36 %
M+2	0.12 % ± 0.04 %	0.44 % ± 0.38 %		4.55 % ± 2.44 %

M+3	0.35 %	±	0.04 %	0.78 %	±	0.37 %				3.29 %	±	1.25 %
M+4	0.09 %	±	0.01 %	0.23 %	±	0.25 %				1.06 %	±	1.06 %
M+5	0.61 %	±	0.07 %	0.24 %	±	0.22 %				2.19 %	±	1.77 %
M+6	10.46 %	±	1.29 %	12.65 %	±	0.83 %				5.18 %	±	1.89 %

Supplemental Table S49 Relative fractions of isotopologues (mol%) of sugars from experiments with *F.* W12-1067 WT grown in medium T supplemented with 11 mM [1,2-$^{13}C_2$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from two independent experiments are shown.

11 mM [1,2-$^{13}C_2$]glucose *F.* W12-1067 WT												
	Glucose in glycogen			Free fructose			Muramic acid			Glucosamine		
M+1	0.00 %	±	0.00 %	0.00 %	±	0.00 %	2.45 %	±	1.63 %	0.92 %	±	0.65 %
M+2	12.46 %	±	0.27 %	11.53 %	±	1.18 %	19.58 %	±	1.72 %	14.51 %	±	0.45 %
M+3	0.00 %	±	0.00 %	1.14 %	±	0.58 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %
M+4	0.00 %	±	0.01 %	0.41 %	±	0.15 %	1.66 %	±	0.55 %	0.01 %	±	0.03 %
M+5	0.00 %	±	0.00 %	0.03 %	±	0.07 %	0.06 %	±	0.20 %	0.33 %	±	0.05 %
M+6	0.02 %	±	0.01 %	0.00 %	±	0.00 %	0.10 %	±	0.13 %	0.00 %	±	0.00 %

Supplemental Table S50 Relative fractions of isotopologues (mol%) of sugars from experiments with *F.* W12-1067 WT grown in medium T supplemented with 3 mM [U-$^{13}C_3$]serine. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

3 mM [U-$^{13}C_3$]serine *F.* W12-1067 WT

	Glucose in glycogen			Free fructose			Muramic acid			Glucosamine		
M+1	0.26 %	±	0.46 %	0.00 %	±	0.00 %	2.44 %	±	2.28 %	0.47 %	±	0.45 %
M+2	0.02 %	±	0.04 %	0.00 %	±	0.00 %	1.16 %	±	2.46 %	0.08 %	±	0.13 %
M+3	0.01 %	±	0.02 %	0.73 %	±	0.47 %	0.61 %	±	1.26 %	0.07 %	±	0.12 %
M+4	0.01 %	±	0.01 %	0.27 %	±	0.26 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %
M+5	0.01 %	±	0.02 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.01 %	±	0.02 %
M+6	0.03 %	±	0.08 %	0.00 %	±	0.00 %	0.13 %	±	0.28 %	0.00 %	±	0.00 %

Supplemental Table S51 Relative fractions of isotopologues (mol%) of sugars from experiments *F.* W12-1067 WT and Δmyo-inositol mutant grown in medium T supplemented with 25 mM [U-$^{13}C_3$]glycerol. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

25 mM [U-$^{13}C_3$]glycerol *F.* W12-1067 WT												
	Glucose in glycogen			Free fructose			Muramic acid			Glucosamine		
M+1	0.00 %	±	0.00 %	0.11 %	±	0.28 %	2.66 %	±	2.90 %	1.63 %	±	0.47 %
M+2	0.02 %	±	0.05 %	0.00 %	±	0.00 %	0.72 %	±	1.28 %	1.21 %	±	0.51 %
M+3	1.08 %	±	0.29 %	0.83 %	±	0.45 %	19.31 %	±	3.83 %	11.45 %	±	2.22 %
M+4	0.02 %	±	0.02 %	0.20 %	±	0.29 %	3.90 %	±	1.34 %	0.40 %	±	0.25 %
M+5	0.02 %	±	0.01 %	0.00 %	±	0.00 %	0.06 %	±	0.15 %	0.80 %	±	0.32 %
M+6	0.06 %	±	0.03 %	0.00 %	±	0.00 %	1.32 %	±	0.75 %	0.58 %	±	0.31 %

25 mM [U-$^{13}C_3$]glycerol Δmyo-inositol mutant									
	Glucose in glycogen			Free fructose			Muramic acid	Glucosamine		
M+1	0.07 %	±	0.14 %	0.00 %	±	0.00 %		1.75 %	±	0.60 %
M+2	0.02 %	±	0.03 %	0.05 %	±	0.08 %		2.40 %	±	0.46 %
M+3	0.80 %	±	0.09 %	0.84 %	±	0.30 %		13.35 %	±	0.71 %
M+4	0.01 %	±	0.01 %	0.18 %	±	0.32 %		0.46 %	±	0.19 %
M+5	0.03 %	±	0.02 %	0.00 %	±	0.00 %		0.94 %	±	0.21 %
M+6	0.15 %	±	0.08 %	0.02 %	±	0.05 %		0.73 %	±	0.24 %

Supplemental Tables S52 ^{13}C-Excess (mol%) of protein-bound amino acids from [U-$^{13}C_6$]glucose liquid medium

^{13}C-Excess (mol%) of protein-bound amino acids from experiments with *NCIB3610*, *GP921* and *GP1562* grown in CSE medium supplemented with 13.4 mM [U-$^{13}C_6$]glucose. Mean and SD from two independent experiments are shown.

	NCIB3610			GP921			GP1562		
Ala	39.04%	±	0.34%	39.14%	±	0.16%	38.26%	±	0.30%
Asp	20.68%	±	0.08%	16.78%	±	0.13%	15.52%	±	0.85%
DAP	29.52%	±	0.23%	27.48%	±	0.24%	26.91%	±	0.87%
Glu	0.26%	±	0.08%	0.93%	±	0.02%	1.16%	±	0.14%
Gly	42.98%	±	0.48%	41.41%	±	0.27%	41.07%	±	0.63%
His	42.93%	±	0.65%	43.61%	±	0.71%	42.19%	±	1.17%
Ile	27.61%	±	0.09%	25.43%	±	0.08%	24.35%	±	0.55%
Leu	38.67%	±	0.07%	38.60%	±	0.11%	37.44%	±	0.12%
Lys	27.89%	±	0.11%	25.59%	±	0.18%	24.56%	±	0.66%
Met	13.23%	±	14.49%	21.77%	±	1.35%	22.19%	±	2.72%
Phe	42.60%	±	0.22%	42.54%	±	0.08%	41.93%	±	0.42%
Pro	0.35%	±	0.08%	0.98%	±	0.07%	1.36%	±	0.22%
Ser	42.62%	±	0.45%	42.68%	±	0.43%	42.31%	±	0.58%
Thr	22.08%	±	0.27%	18.23%	±	0.25%	16.51%	±	0.76%
Tyr	43.33%	±	0.17%	42.98%	±	0.16%	42.43%	±	0.25%
Val	39.02%	±	0.14%	39.14%	±	0.07%	38.05%	±	0.22%

Supplemental Table S53 ^{13}C-Excess (mol%) of polar metabolites from [U-$^{13}C_6$]glucose liquid medium

^{13}C-Excess (mol%) of polar metabolites from experiments with *NCIB3610*, *GP921* and *GP1562* grown in CSE medium supplemented with 13.4 mM [U-$^{13}C_6$]glucose. Mean and SD from two independent experiments are shown.

	NCIB3610			GP921			GP1562		
Lactate	3.33%	±	1.74%	26.55%	±	3.08%	8.77%	±	2.12%
Glycolic acid	14.04%	±	2.95%	19.33%	±	4.46%	16.55%	±	0.33%
Alanine	38.63%	±	0.38%	39.96%	±	0.47%	38.58%	±	0.67%
Glycine	31.57%	±	20.36%	36.67%	±	5.06%	40.30%	±	1.01%
Valine	37.68%	±	1.10%	36.57%	±	0.52%	36.20%	±	0.47%
leucine	37.97%	±	0.43%	38.86%	±	0.37%	38.30%	±	0.34%
Isoleucine	26.64%	±	0.72%	26.67%	±	0.21%	23.79%	±	0.76%
Succinic acid	0.64%	±	0.03%	2.21%	±	0.14%	1.58%	±	1.09%
Fumaric acid	4.72%	±	2.83%	11.12%	±	0.36%	4.33%	±	0.52%
Glycerol	7.23%	±	1.35%	21.36%	±	3.77%	5.40%	±	0.68%
Methionine	19.10%	±	7.60%	24.07%	±	0.54%	21.22%	±	1.08%

Serine	39.19%	±	1.45%	42.48%	±	0.71%	43.01%	±	0.80%
Threonine	19.89%	±	0.95%	18.61%	±	0.99%	15.57%	±	1.70%
Phenylalanine	41.75%	±	0.72%	41.76%	±	0.46%	42.13%	±	1.21%
Malic acid	16.82%	±	19.27%	9.44%	±	2.55%	4.07%	±	2.61%
Aspartate	15.83%	±	0.90%	14.78%	±	2.03%	14.15%	±	2.03%
Glutamate	0.40%	±	0.73%	0.79%	±	0.06%	0.99%	±	0.20%
Palmitic acid	7.60%	±	1.73%	6.46%	±	0.24%	3.74%	±	0.23%
Stearic acid	1.36%	±	0.41%	1.41%	±	0.24%	2.91%	±	1.92%
Citric acid	16.03%	±	32.25%	0.62%	±	0.14%	0.42%	±	0.22%

Supplemental Table S54 ^{13}C-Excess (mol%) of sugars from [U-$^{13}C_6$]glucose liquid medium

^{13}C-Excess (mol%) of sugars from experiments with *NCIB3610*, *GP921* and *GP1562* grown in CSE medium supplemented with 13.4 mM [U-$^{13}C_6$]glucose. Mean and SD from two independent experiments are shown.

	NCIB3610			GP921			GP1562		
Glucose in glycogen	40.80%	±	1.27%	39.71%	±	1.42%	40.28%	±	1.59%
Glucosamine	43.06%	±	1.37%	41.88%	±	1.15%	39.82%	±	4.33%
Muramic acid	42.97%	±	1.29%	41.03%	±	1.28%	42.01%	±	2.49%

Supplemental Tables S55 ^{13}C-Excess (mol%) of protein-bound amino acids from [U-$^{13}C_6$]glucose agar plate

^{13}C-Excess (mol%) of protein-bound amino acids from experiments with *NCIB3610*, *GP921* and *GP1562* grown on Msgg agar plate supplemented with 9 mM [U-$^{13}C_6$]glucose. Mean and SD from six independent experiments are shown.

	NCIB3610			GP921			GP1562		
Ala	16.67%	±	1.39%	23.63%	±	1.22%	30.09%	±	0.98%
Asp	9.17%	±	0.30%	12.63%	±	0.51%	12.09%	±	0.37%
DAP	12.90%	±	0.74%	16.29%	±	1.03%	18.98%	±	0.61%
Glu	8.22%	±	0.18%	3.18%	±	0.26%	8.73%	±	0.22%
Gly	22.26%	±	1.52%	20.64%	±	1.11%	36.45%	±	0.75%
His	30.62%	±	1.04%	21.71%	±	1.21%	42.38%	±	0.81%
Ile	12.72%	±	0.46%	16.02%	±	0.56%	18.71%	±	0.63%
Leu	21.33%	±	0.76%	25.37%	±	0.62%	30.76%	±	1.81%
Lys	15.28%	±	0.40%	18.29%	±	0.65%	19.27%	±	0.61%
Met	15.05%	±	2.50%	16.77%	±	4.21%	19.74%	±	3.10%
Phe	1.87%	±	0.27%	0.58%	±	0.38%	0.88%	±	0.17%
Pro	7.01%	±	0.58%	2.16%	±	0.37%	8.36%	±	0.59%
Ser	18.55%	±	2.11%	18.66%	±	1.48%	33.76%	±	1.55%
Thr	9.03%	±	0.39%	12.58%	±	1.72%	11.82%	±	0.37%

Tyr	25.07%	±	1.01%	22.16%	±	1.32%	36.98%	±	0.68%
Val	18.58%	±	1.17%	24.00%	±	0.98%	30.28%	±	1.14%

Supplemental Table S56 ^{13}C-Excess (mol%) of polar metabolites from [U-$^{13}C_6$]glucose agar plate

^{13}C-Excess (mol%) of polar metabolites from experiments with *NCIB3610*, *GP921* and *GP1562* grown on Msgg agar plate supplemented with 9 mM [U-$^{13}C_6$]glucose. Mean and SD from six independent experiments are shown.

	NCIB3610			GP921			GP1562		
Lactate	2.17%	±	0.70%	1.64%	±	1.36%	0.87%	±	0.35%
Glycolic acid	7.45%	±	0.77%	4.59%	±	1.67%	7.54%	±	2.18%
Alanine	7.59%	±	0.39%	15.96%	±	4.46%	15.50%	±	3.98%
Glycine	13.08%	±	0.85%	9.06%	±	1.50%	26.02%	±	2.53%
Oxalic acid	5.06%	±	2.07%	5.91%	±	6.29%	8.53%	±	6.87%
Valine	10.64%	±	0.58%	17.67%	±	0.90%	21.01%	±	4.57%
leucine	18.25%	±	0.76%	20.68%	±	1.45%	26.57%	±	3.50%
Isoleucine	10.49%	±	0.43%	12.33%	±	2.18%	15.43%	±	2.10%
Succinic acid	5.31%	±	1.48%	2.82%	±	0.48%	8.13%	±	3.91%
Fumaric acid	8.36%	±	0.21%	8.55%	±	0.75%	9.59%	±	0.76%
Glycerol	1.12%	±	0.59%	1.14%	±	0.64%	1.78%	±	1.24%
Methionine	14.26%	±	0.75%	14.02%	±	1.33%	23.02%	±	7.41%
Serine	8.16%	±	1.14%	9.18%	±	1.98%	15.38%	±	4.83%
Threonine	12.53%	±	7.00%	32.20%	±	2.67%	19.30%	±	10.94%
Phenylalanine	2.44%	±	0.84%	3.93%	±	1.23%	3.18%	±	1.68%
Malic acid	6.68%	±	1.40%	7.58%	±	3.42%	26.69%	±	21.31%
Aspartate	8.07%	±	0.74%	7.28%	±	4.30%	9.96%	±	4.27%
Glutamate	8.26%	±	0.39%	4.07%	±	0.62%	8.66%	±	0.32%
Oelic acid	2.65%	±	1.01%	3.28%	±	2.87%	6.42%	±	7.42%
Stearic acid	0.23%	±	0.06%	0.21%	±	0.07%	0.10%	±	0.04%
Citric acid				5.70%	±	1.46%	12.41%	±	3.60%

Supplemental Table S57 ^{13}C-Excess (mol%) of sugars from [U-$^{13}C_6$]glucose agar plate

^{13}C-Excess (mol%) of sugars from experiments with *NCIB3610*, *GP921* and *GP1562* grown on Msgg agar plate supplemented with 9 mM [U-$^{13}C_6$]glucose. Mean and SD from six independent experiments are shown.

	NCIB3610			GP921			GP1562		
Glucose in glycogen	19.59%	±	2.04%	19.15%	±	5.11%	39.79%	±	5.69%
Glucosamine	15.45%	±	2.33%	20.62%	±	5.31%	36.39%	±	5.96%
Muramic acid	16.53%	±	5.16%	16.84%	±	2.34%	34.67%	±	7.49%

Supplemental Tables S58 ^{13}C-Excess (mol%) of protein-bound amino acids from [U-$^{13}C_5$]gltamate agar plate

^{13}C-Excess (mol%) of protein-bound amino acids from experiments with *NCIB3610*, *GP921* and *GP1562* grown on Msgg agar plate supplemented with 6 mM [U-$^{13}C_5$]glutamate. Mean and SD from three independent experiments are shown.

	NCIB			GP921			GP1562		
Ala	5.15%	±	0.12%	6.84%	±	0.12%	6.38%	±	0.10%
Asp	11.90%	±	0.30%	13.45%	±	0.22%	14.51%	±	0.19%
DAP	9.13%	±	0.31%	10.40%	±	0.11%	10.82%	±	0.10%
Glu	13.43%	±	0.47%	16.89%	±	0.31%	16.57%	±	0.28%
Gly	2.78%	±	0.07%	1.81%	±	0.14%	2.78%	±	0.08%
His	0.58%	±	0.79%	0.14%	±	0.11%	0.57%	±	0.08%
Ile	10.01%	±	0.17%	10.31%	±	0.15%	10.98%	±	0.15%
Leu	5.20%	±	0.15%	6.00%	±	0.06%	6.08%	±	0.06%
Lys	10.19%	±	0.22%	9.13%	±	0.08%	10.93%	±	0.16%
Met	9.92%	±	0.24%	9.06%	±	0.26%	10.52%	±	0.23%
Phe	0.83%	±	0.03%	0.13%	±	0.04%	0.37%	±	0.05%
Pro	13.53%	±	0.67%	15.27%	±	0.26%	14.46%	±	0.20%
Ser	2.35%	±	0.13%	1.40%	±	0.12%	2.43%	±	0.21%
Thr	12.99%	±	0.12%	13.29%	±	0.11%	14.57%	±	0.12%
Tyr	1.69%	±	0.10%	1.12%	±	0.05%	2.38%	±	0.04%
Val	4.93%	±	0.16%	6.28%	±	0.11%	5.77%	±	0.13%

Supplemental Table S59 ^{13}C-Excess (mol%) of polar metabolites from [U-$^{13}C_5$]glutamate agar plate

^{13}C-Excess (mol%) of polar metabolites from experiments with *NCIB3610*, *GP921* and *GP1562* grown on Msgg agar plate supplemented with 6 mM [U-$^{13}C_5$]glutamate. Mean and SD from three independent experiments are shown.

	NCIB			GP921			GP1562		
Lactate	2.84%	±	0.48%	2.72%	±	0.16%	0.42%	±	0.03%
Glycolic acid	0.48%	±	0.27%	0.29%	±	0.18%	0.31%	±	0.25%
Alanine	5.93%	±	0.32%	5.96%	±	0.26%	4.07%	±	0.31%
Glycine	3.64%	±	0.30%	1.50%	±	0.18%	1.44%	±	0.26%
Valine	4.64%	±	0.19%	6.79%	±	0.15%	4.60%	±	0.13%
leucine	4.83%	±	0.18%	6.53%	±	0.15%	5.04%	±	0.36%
Isoleucine	9.97%	±	0.16%	10.78%	±	0.38%	10.47%	±	0.27%
Succinic acid	14.94%	±	0.89%						
Fumaric acid	11.47%	±	0.36%	13.56%	±	0.34%	13.33%	±	0.29%
Glycerol	0.55%	±	0.15%	0.18%	±	0.18%	0.24%	±	0.19%
Methionine	8.12%	±	0.29%	8.09%	±	0.34%	10.17%	±	0.50%

Serine	4.77%	±	0.24%	1.98%	±	0.22%	1.15%	±	0.15%
Threonine	12.42%	±	0.19%	12.89%	±	0.50%	13.60%	±	0.51%
Phenylalanine	1.09%	±	0.10%	0.34%	±	0.08%	0.55%	±	0.22%
Malic acid	12.28%	±	0.94%	14.76%	±	0.34%	10.94%	±	1.23%
Aspartate	10.88%	±	0.30%	14.76%	±	0.35%	13.34%	±	0.39%
Glutamate	10.51%	±	0.47%	15.40%	±	0.36%	14.02%	±	0.28%
Palmitate	1.80%	±	0.39%	0.63%	±	0.09%	0.22%	±	0.06%
Oelic acid	4.67%	±	0.27%	4.40%	±	0.21%	3.81%	±	0.35%
Stearic acid	4.29%	±	1.62%	0.80%	±	0.20%	0.44%	±	0.13%
Citric acid				0.00%	±	0.00%	0.00%	±	0.00%

Supplemental Table S60 ^{13}C-Excess (mol%) of sugars from [U-$^{13}C_5$]glutamate agar plate

^{13}C-Excess (mol%) of sugars from experiments with *NCIB3610*, *GP921* and *GP1562* grown on Msgg agar plate supplemented with 6 mM [U-$^{13}C_5$]glutamate. Mean and SD from three independent experiments are shown.

	NCIB			GP921			GP1562		
Glucose in glycogen	0.72%	±	0.12%	0.52%	±	0.63%	0.43%	±	0.26%
Glucosamine	1.01%	±	0.58%	0.22%	±	0.15%	0.42%	±	0.25%
Muramic acid	2.81%	±	0.72%	1.48%	±	0.28%	2.27%	±	0.48%

Supplemental Table S61 Relative fractions of isotopologues (mol%) of amino acids from experiments with *NCIB3610*, *GP921* and *GP1562* grown in CSE medium supplemented with 13.4 mM [U-$^{13}C_6$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from two independent experiments are shown.

13.4 mM [U-$^{13}C_6$]glucose *NCIB3610*								
	Ala	Asp	DAP	Glu	Gly	His	Ile	Leu
M+1	3.25 % ± 0.27 %	11.16 % ± 0.19 %	9.04 % ± 0.64 %	0.03 % ± 0.06 %	6.70 % ± 0.16 %	21.50 % ± 1.44 %	5.94 % ± 0.14 %	14.58 % ± 0.15 %
M+2	3.65 % ± 0.13 %	1.89 % ± 0.11 %	4.34 % ± 0.27 %	0.30 % ± 0.27 %	39.63 % ± 0.58 %	5.81 % ± 1.41 %	35.44 % ± 0.26 %	29.28 % ± 0.16 %
M+3	35.52 % ± 0.34 %	15.07 % ± 0.15 %	32.86 % ± 0.49 %	0.11 % ± 0.05 %	±	8.59 % ± 1.29 %	7.99 % ± 0.21 %	18.13 % ± 0.08 %
M+4		5.64 % ± 0.09 %	8.48 % ± 0.45 %	0.02 % ± 0.02 %		5.87 % ± 0.83 %	5.75 % ± 0.08 %	9.39 % ± 0.09 %
M+5			1.25 % ± 0.37 %	0.05 % ± 0.01 %		19.99 % ± 0.81 %	2.85 % ± 0.05 %	5.65 % ± 0.03 %
M+6			5.90 % ± 0.25 %			12.55 % ± 0.95 %		
M+7			2.11 % ± 0.15 %					

13.4 mM [U-$^{13}C_6$]glucose *NCIB3610*								
	Lys	Met	Phe	Pro	Ser	Thr	Tyr	Val
M+	7.45 ± 0.21	31.96 ± 0.35	4.03 ± 0.09	0.18 ± 0.20	4.27 ± 0.46	12.90 ± 0.52	3.57 ± 0.10	2.66 ± 0.08

1	%		%	%		%	%		%	%		%	%		%	%		%	%		%
M+2	18.84 %	±	0.23 %	5.34 %	±	0.15 %	11.74 %	±	0.24 %	0.00 %	±	0.00 %	5.20 %	±	0.18 %	2.35 %	±	0.30 %	12.29 %	±	0.10 %	24.35 %	±	0.19 %
M+3	21.50 %	±	0.37 %	10.12 %	±	0.20 %	17.47 %	±	0.40 %	0.32 %	±	0.04 %	37.73 %	±	0.38 %	16.35 %	±	0.28 %	16.12 %	±	0.26 %	22.85 %	±	0.19 %
M+4	4.02 %	±	0.12 %	10.77 %	±	0.11 %	13.35 %	±	0.19 %	0.14 %	±	0.11 %				5.42 %	±	0.08 %	14.00 %	±	0.26 %	1.72 %	±	0.07 %
M+5	5.61 %	±	0.17 %	3.24 %	±	0.07 %	11.07 %	±	0.29 %	0.00 %	±	0.00 %							11.34 %	±	0.27 %	13.66 %	±	0.08 %
M+6	2.26 %	±	0.04 %		±		11.62 %	±	0.28 %										12.21 %	±	0.20 %			
M+7							8.41 %	±	0.15 %										8.32 %	±	0.16 %			
M+8							2.00 %	±	0.09 %										2.24 %	±	0.16 %			
M+9							5.57 %	±	0.07 %										5.70 %	±	0.14 %			

13.4 mM [U-$^{13}C_6$]glucose *GP921*																								
	Ala			Asp			DAP			Glu			Gly			His			Ile			Leu		
M+1	2.87 %	±	0.21 %	8.69 %	±	0.21 %	7.48 %	±	0.34 %	0.16 %	±	0.23 %	6.11 %	±	0.10 %	20.29 %	±	1.91 %	4.75 %	±	0.16 %	14.74 %	±	0.12 %
M+2	3.41 %	±	0.05 %	2.31 %	±	0.12 %	3.99 %	±	0.54 %	1.07 %	±	0.13 %	38.36 %	±	0.30 %	7.47 %	±	1.23 %	35.89 %	±	0.18 %	28.90 %	±	0.16 %

M+3	35.91 % ± 0.15 %	12.61 % ± 0.18 %	33.95 % ± 0.60 %	0.38 % ± 0.07 %		10.33 % ± 0.54 %	6.53 % ± 0.12 %	18.27 % ± 0.10 %
M+4		3.99 % ± 0.07 %	6.34 % ± 0.26 %	0.17 % ± 0.02 %		6.30 % ± 0.80 %	4.67 % ± 0.10 %	9.39 % ± 0.05 %
M+5			1.47 % ± 0.24 %	0.11 % ± 0.01 %		20.43 % ± 0.88 %	2.47 % ± 0.04 %	5.62 % ± 0.07 %
M+6			5.05 % ± 0.29 %			11.35 % ± 1.36 %		
M+7			1.72 % ± 0.19 %					

13.4 mM [U-$^{13}C_6$]glucose *GP921*								
	Lys	Met	Phe	Pro	Ser	Thr	Tyr	Val
M+1	6.24 % ± 0.25 %	30.33 % ± 4.07 %	4.10 % ± 0.18 %	0.61 % ± 0.24 %	3.84 % ± 0.28 %	10.87 % ± 0.55 %	3.72 % ± 0.19 %	2.75 % ± 0.10 %
M+2	19.27 % ± 0.29 %	5.34 % ± 2.77 %	11.52 % ± 0.09 %	0.56 % ± 0.22 %	4.61 % ± 0.10 %	2.90 % ± 0.23 %	12.37 % ± 0.35 %	24.09 % ± 0.11 %
M+3	20.44 % ± 0.30 %	9.69 % ± 2.36 %	17.89 % ± 0.27 %	0.61 % ± 0.05 %	38.32 % ± 0.39 %	13.40 % ± 0.22 %	16.59 % ± 0.36 %	23.03 % ± 0.09 %
M+4	3.34 % ± 0.20 %	8.39 % ± 0.51 %	13.28 % ± 0.14 %	0.32 % ± 0.09 %		4.02 % ± 0.17 %	13.89 % ± 0.45 %	1.70 % ± 0.10 %
M+5	4.62 % ± 0.24 %	1.04 % ± 1.14 %	10.95 % ± 0.17 %	0.01 % ± 0.02 %			10.83 % ± 0.45 %	13.78 % ± 0.07 %
M+	1.83 ± 0.14		11.70 ± 0.27				12.09 ± 0.22	

6	% %		% %				% %	
M+7			8.18 % ± 0.17 %				8.32 % ± 0.34 %	
M+8			2.16 % ± 0.11 %				2.23 % ± 0.20 %	
M+9			5.49 % ± 0.13 %				5.59 % ± 0.32 %	

13.4 mM [U-$^{13}C_6$]glucose *GP1562*								
	Ala	Asp	DAP	Glu	Gly	His	Ile	Leu
M+1	3.18 % ± 0.16 %	7.81 % ± 0.33 %	7.26 % ± 0.59 %	0.10 % ± 0.10 %	6.46 % ± 0.50 %	20.54 % ± 1.40 %	4.69 % ± 0.12 %	14.60 % ± 0.36 %
M+2	3.56 % ± 0.26 %	2.45 % ± 0.19 %	4.54 % ± 0.54 %	1.61 % ± 0.23 %	37.84 % ± 0.45 %	6.87 % ± 0.61 %	35.42 % ± 0.13 %	28.67 % ± 0.26 %
M+3	34.82 % ± 0.53 %	11.55 % ± 0.76 %	33.21 % ± 1.02 %	0.37 % ± 0.05 %		9.58 % ± 0.98 %	5.90 % ± 0.45 %	17.58 % ± 0.12 %
M+4		3.68 % ± 0.37 %	6.26 % ± 0.69 %	0.18 % ± 0.04 %		5.94 % ± 0.40 %	4.46 % ± 0.17 %	8.99 % ± 0.16 %
M+5			1.40 % ± 0.47 %	0.12 % ± 0.01 %		18.90 % ± 1.69 %	2.13 % ± 0.25 %	5.31 % ± 0.17 %
M+6			4.82 % ± 0.47 %			11.98 % ± 0.43 %		
M+7			1.64 % ± 0.25 %					

13.4 mM [U-$^{13}C_6$]glucose *GP1562*								
	Lys	Met	Phe	Pro	Ser	Thr	Tyr	Val
M+1	6.20 % ± 0.51 %	28.83 % ± 7.11 %	4.07 % ± 0.09 %	0.52 % ± 0.26 %	4.49 % ± 0.41 %	9.46 % ± 0.65 %	3.69 % ± 0.14 %	2.90 % ± 0.26 %
M+2	19.01 % ± 0.26 %	7.47 % ± 2.08 %	11.50 % ± 0.26 %	1.22 % ± 0.26 %	5.02 % ± 0.40 %	3.32 % ± 0.28 %	12.18 % ± 0.17 %	23.74 % ± 0.14 %
M+3	20.10 % ± 0.52 %	6.16 % ± 5.51 %	17.23 % ± 0.16 %	0.68 % ± 0.09 %	37.46 % ± 0.29 %	12.16 % ± 0.68 %	16.10 % ± 0.31 %	22.55 % ± 0.16 %
M+4	3.01 % ± 0.23 %	6.99 % ± 1.32 %	13.27 % ± 0.43 %	0.37 % ± 0.15 %		3.36 % ± 0.34 %	13.82 % ± 0.17 %	1.76 % ± 0.18 %
M+5	4.14 % ± 0.34 %	4.15 % ± 3.69 %	10.65 % ± 0.32 %	0.06 % ± 0.05 %			10.81 % ± 0.28 %	13.04 % ± 0.37 %
M+6	1.68 % ± 0.22 %		11.63 % ± 0.23 %				11.98 % ± 0.33 %	
M+7			8.33 % ± 0.19 %				8.26 % ± 0.26 %	
M+8			2.00 % ± 0.12 %				2.16 % ± 0.23 %	
M+9			5.36 % ± 0.09 %				5.47 % ± 0.19 %	

Supplemental Table S62 Relative fractions of isotopologues (mol%) of polar metabolites from experiments with *NCIB3610*, *GP921* and *GP1562* grown in CSE medium supplemented with 13.4 mM [U-$^{13}C_6$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from two independent experiments are shown.

13.4 mM [U-$^{13}C_6$]glucose *NCIB3610*								
	Lactate	Glycolic acid	Alanine	Glycine	Valine	leucine	Isoleucine	Succinic acid
M+1	0.00 % ± 0.01 %	2.07 % ± 1.54 %	3.13 % ± 0.48 %	0.91 % ± 1.01 %	2.74 % ± 0.95 %	15.48 % ± 1.54 %	8.15 % ± 2.14 %	0.00 % ± 0.00 %
M+2	0.31 % ± 0.30 %	13.01 % ± 2.53 %	3.57 % ± 0.36 %	31.12 % ± 22.78 %	22.61 % ± 0.63 %	24.89 % ± 3.68 %	33.66 % ± 1.50 %	0.00 % ± 0.00 %
M+3	3.12 % ± 1.70 %		35.21 % ± 0.48 %		21.65 % ± 0.49 %	17.20 % ± 0.16 %	9.95 % ± 1.76 %	0.71 % ± 0.03 %
M+4					2.56 % ± 1.03 %	9.10 % ± 0.41 %	4.60 % ± 1.08 %	0.11 % ± 0.04 %
M+5					13.05 % ± 0.70 %	7.33 % ± 1.71 %	1.90 % ± 0.96 %	

13.4 mM [U-$^{13}C_6$]glucose *NCIB3610*								
	Fumaric acid	Glycerol	Methionine	Serine	Threonine	Phenylalanine	Malic acid	Aspartate
M+1	0.72 % ± 1.15 %	0.79 % ± 0.66 %	26.01 % ± 7.80 %	5.40 % ± 1.98 %	9.06 % ± 2.35 %	3.37 % ± 1.24 %	18.98 % ± 24.87 %	7.63 % ± 4.11 %
M+2	0.08 % ± 0.19 %	0.39 % ± 0.30 %	2.88 % ± 3.32 %	5.59 % ± 0.49 %	2.12 % ± 1.26 %	14.35 % ± 3.35 %	11.53 % ± 17.86 %	2.39 % ± 2.23 %
M+3	4.05 % ± 2.45 %	6.71 % ± 1.40 %	8.81 % ± 1.86 %	33.66 % ± 1.00 %	15.95 % ± 0.36 %	17.08 % ± 0.82 %	1.53 % ± 3.69 %	13.36 % ± 1.32 %
M+	1.46 ± 1.60		6.84 ± 3.62		4.60 ± 0.87	12.61 ± 1.00	5.16 ± 6.73	2.71 ± 1.31

4	% %		% %		% %	% %	% %	% %	
M+5			1.98 % ± 1.81 %			10.77 % ± 1.15 %			
M+6						11.53 % ± 0.86 %			
M+7						6.85 % ± 2.87 %			
M+8						2.69 % ± 0.83 %			
M+9						5.51 % ± 1.26 %			

13.4 mM [U-$^{13}C_6$]glucose *NCIB3610*					
	Glutamate	Palmitate	Oelic acid	Stearic acid	Citric acid
M+1	1.03 % ± 2.53 %	0.00 % ± 0.00 %		0.27 % ± 0.49 %	0.83 % ± 2.03 %
M+2	0.00 % ± 0.00 %	1.46 % ± 0.60 %		0.22 % ± 0.24 %	0.22 % ± 0.54 %
M+3	0.04 % ± 0.05 %	0.29 % ± 0.09 %		0.10 % ± 0.14 %	0.56 % ± 0.91 %
M+4	0.00 % ± 0.00 %	4.30 % ± 1.06 %		0.50 % ± 0.24 %	0.98 % ± 1.62 %
M+5	0.17 % ± 0.30 %	0.37 % ± 0.17 %		0.05 % ± 0.06 %	0.00 % ± 0.00 %
M+6		5.44 % ± 1.41 %		0.76 % ± 0.36 %	14.89 % ± 33.79 %
M+7		0.27 % ± 0.11 %		0.08 % ± 0.08 %	

M+8		4.08 % ± 1.06 %		0.68 % ± 0.23 %	
M+9		0.23 % ± 0.09 %		0.10 % ± 0.08 %	
M+10		1.99 % ± 0.41 %		0.37 % ± 0.12 %	
M+11		0.08 % ± 0.05 %		0.07 % ± 0.05 %	
M+12		0.60 % ± 0.15 %		0.13 % ± 0.06 %	
M+13		0.01 % ± 0.01 %		0.07 % ± 0.05 %	
M+14		0.08 % ± 0.06 %		0.04 % ± 0.06 %	
M+15		0.00 % ± 0.00 %		0.02 % ± 0.03 %	
M+16		0.02 % ± 0.03 %		0.06 % ± 0.05 %	
M+17				0.02 % ± 0.03 %	
M+18				0.04 % ± 0.03 %	

13.4 mM [U-$^{13}C_6$]glucose *GP921*								
	Lactate	Glycolic acid	Alanine	Glycine	Valine	leucine	Isoleucine	Succinic acid
M+1	3.02 % ± 0.70 %	3.60 % ± 1.11 %	3.68 % ± 0.28 %	5.83 % ± 1.57 %	2.57 % ± 0.36 %	14.45 % ± 0.21 %	6.15 % ± 0.42 %	0.00 % ± 0.00 %
M+2	3.73 % ± 0.60 %	17.53 % ± 4.33 %	3.98 % ± 0.27 %	33.75 % ± 4.77 %	23.68 % ± 0.48 %	29.42 % ± 0.47 %	35.33 % ± 0.36 %	0.57 % ± 0.23 %
M+3	23.06 % ± 2.74 %		36.09 % ± 0.51 %		21.76 % ± 0.61 %	18.24 % ± 0.49 %	7.64 % ± 0.27 %	1.87 % ± 0.17 %

M+4					1.63 % ± 0.15 %	9.47 % ± 0.15 %	5.01 % ± 0.11 %	0.52 % ± 0.09 %
M+5					12.22 % ± 0.38 %	5.68 % ± 0.21 %	2.71 % ± 0.19 %	

13.4 mM [U-$^{13}C_6$]glucose *GP921*								
	Fumaric acid	Glycerol	Methionine	Serine	Threonine	Phenylalanine	Malic acid	Aspartate
M+1	3.79 % ± 0.43 %	2.09 % ± 1.05 %	35.19 % ± 0.92 %	6.70 % ± 1.43 %	8.81 % ± 2.36 %	5.15 % ± 0.79 %	3.96 % ± 3.22 %	6.95 % ± 3.80 %
M+2	1.80 % ± 0.16 %	2.49 % ± 0.73 %	4.61 % ± 0.50 %	7.51 % ± 0.84 %	2.70 % ± 0.78 %	11.54 % ± 0.93 %	1.84 % ± 1.59 %	2.41 % ± 0.75 %
M+3	8.72 % ± 0.13 %	19.01 % ± 3.54 %	8.18 % ± 0.80 %	35.24 % ± 0.67 %	13.62 % ± 1.71 %	17.78 % ± 0.52 %	6.30 % ± 1.84 %	11.14 % ± 2.79 %
M+4	2.73 % ± 0.17 %		9.07 % ± 0.51 %		4.84 % ± 0.91 %	13.29 % ± 0.70 %	2.80 % ± 1.72 %	3.48 % ± 0.95 %
M+5			3.03 % ± 0.33 %			10.70 % ± 0.45 %		
M+6						11.64 % ± 0.79 %		
M+7						7.76 % ± 0.56 %		
M+8						2.18 % ± 0.37 %		
M+						5.11 ± 0.49		

9						% %		

13.4 mM [U-$^{13}C_6$]glucose *GP921*					
	Glutamate	Palmitate	Oelic acid	Stearic acid	Citric acid
M+1	0.00 % ± 0.01 %	0.00 % ± 0.00 %		0.56 % ± 0.58 %	0.66 % ± 1.03 %
M+2	1.14 % ± 0.17 %	1.32 % ± 0.29 %		0.21 % ± 0.30 %	0.29 % ± 0.36 %
M+3	0.24 % ± 0.09 %	0.30 % ± 0.15 %		0.04 % ± 0.05 %	0.35 % ± 0.36 %
M+4	0.09 % ± 0.03 %	3.55 % ± 0.10 %		0.50 % ± 0.08 %	0.20 % ± 0.19 %
M+5	0.11 % ± 0.01 %	0.39 % ± 0.19 %		0.07 % ± 0.07 %	0.09 % ± 0.11 %
M+6		4.53 % ± 0.26 %		0.72 % ± 0.16 %	0.03 % ± 0.05 %
M+7		0.30 % ± 0.09 %		0.11 % ± 0.08 %	
M+8		3.44 % ± 0.22 %		0.65 % ± 0.13 %	
M+9		0.17 % ± 0.13 %		0.08 % ± 0.07 %	
M+10		1.69 % ± 0.15 %		0.42 % ± 0.12 %	
M+11		0.08 % ± 0.05 %		0.10 % ± 0.08 %	
M+12		0.45 % ± 0.04 %		0.16 % ± 0.03 %	
M+13		0.05 % ± 0.03 %		0.02 % ± 0.02 %	
M+14		0.05 % ± 0.06 %		0.11 % ± 0.08 %	
M+15		0.02 % ± 0.03 %		0.04 % ± 0.06 %	

M+16		0.02 % ± 0.03 %		0.05 % ± 0.05 %	
M+17				0.01 % ± 0.03 %	
M+18				0.02 % ± 0.03 %	

13.4 mM [U-$^{13}C_6$]glucose *GP1562*								
	Lactate	Glycolic acid	Alanine	Glycine	Valine	leucine	Isoleucine	Succinic acid
M+1	0.07 % ± 0.11 %	4.97 % ± 0.88 %	3.16 % ± 0.21 %	6.25 % ± 0.69 %	2.72 % ± 0.50 %	15.28 % ± 1.17 %	5.34 % ± 0.67 %	0.00 % ± 0.00 %
M+2	0.97 % ± 0.15 %	14.07 % ± 0.31 %	3.36 % ± 0.27 %	37.17 % ± 0.95 %	23.44 % ± 0.93 %	29.29 % ± 0.46 %	35.22 % ± 1.18 %	0.06 % ± 0.10 %
M+3	8.10 % ± 2.23 %		35.29 % ± 0.76 %		21.73 % ± 0.85 %	18.12 % ± 0.80 %	5.55 % ± 0.49 %	1.60 % ± 1.13 %
M+4					1.50 % ± 0.56 %	8.70 % ± 0.65 %	3.98 % ± 0.28 %	0.35 % ± 0.31 %
M+5					12.04 % ± 0.27 %	5.70 % ± 0.45 %	2.12 % ± 0.37 %	

13.4 mM [U-$^{13}C_6$]glucose *GP1562*								
	Fumaric acid	Glycerol	Methionine	Serine	Threonine	Phenylalanine	Malic acid	Aspartate
M+1	0.55 % ± 1.28 %	0.93 % ± 0.94 %	35.61 % ± 1.95 %	5.44 % ± 1.99 %	6.99 % ± 3.16 %	4.87 % ± 1.31 %	1.04 % ± 2.54 %	8.01 % ± 3.84 %
M+	0.76 ± 0.40	0.50 ± 0.49	4.55 ± 2.63	5.52 ± 1.60	2.72 ± 1.81	11.20 ± 1.17	2.74 ± 3.75	2.02 ± 2.87

2	% %	% %	% %	% %	% %	% %	% %	% %	% %	% %
M+3	3.44 % ± 0.30 %	4.76 % ± 0.67 %	7.75 % ± 1.75 %	37.52 % ± 1.50 %	11.64 % ± 1.74 %	16.54 % ± 2.02 %	2.61 % ± 2.65 %	10.36 % ± 4.38 %		
M+4	1.23 % ± 0.34 %		5.70 % ± 2.84 %		3.73 % ± 1.41 %	13.54 % ± 0.93 %	0.49 % ± 0.54 %	3.37 % ± 4.59 %		
M+5			3.06 % ± 1.51 %			10.76 % ± 0.70 %				
M+6						10.80 % ± 1.21 %				
M+7						9.59 % ± 0.77 %				
M+8						1.69 % ± 0.91 %				
M+9						5.43 % ± 0.75 %				

13.4 mM [U-$^{13}C_6$]glucose *GP1562*					
	Glutamate	Palmitate	Oelic acid	Stearic acid	Citric acid
M+1	0.20 % ± 0.40 %	0.00 % ± 0.00 %		0.00 % ± 0.76 %	0.00 % ± 0.00 %
M+2	1.49 % ± 0.35 %	0.31 % ± 0.30 %		0.30 % ± 0.52 %	0.14 % ± 0.30 %
M+3	0.27 % ± 0.08 %	0.29 % ± 0.25 %		0.25 % ± 0.39 %	0.45 % ± 0.56 %
M+4	0.13 % ± 0.11 %	2.15 % ± 0.34 %		0.34 % ± 0.38 %	0.08 % ± 0.17 %

M+5	0.08 % ± 0.03 %	0.33 % ± 0.24 %		0.24 % ± 0.24 %	0.06 % ± 0.14 %
M+6		2.55 % ± 0.19 %		0.19 % ± 0.23 %	0.04 % ± 0.06 %
M+7		0.24 % ± 0.18 %		0.18 % ± 0.43 %	
M+8		1.89 % ± 0.32 %		0.32 % ± 0.29 %	
M+9		0.18 % ± 0.21 %		0.21 % ± 0.49 %	
M+10		0.93 % ± 0.18 %		0.18 % ± 0.30 %	
M+11		0.04 % ± 0.06 %		0.06 % ± 0.05 %	
M+12		0.21 % ± 0.19 %		0.19 % ± 0.42 %	
M+13		0.03 % ± 0.04 %		0.04 % ± 0.16 %	
M+14		0.04 % ± 0.03 %		0.03 % ± 1.07 %	
M+15		0.03 % ± 0.05 %		0.05 % ± 0.40 %	
M+16		0.04 % ± 0.04 %		0.04 % ± 0.51 %	
M+17				0.00 % ± 0.21 %	
M+18				0.30 % ± 0.26 %	

Supplemental Table S63 Relative fractions of isotopologues (mol%) of sugars from experiments with *NCIB3610*, *GP921* and *GP1562* grown in CSE medium supplemented with 13.4 mM [U-$^{13}C_6$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from two independent experiments are shown.

13.4 mM [U-$^{13}C_6$]glucose *NCIB3610*

	Glucose in glycogen	Glucosamine	Muramic acid
M+1	1.15 % ± 0.35 %	3.24 % ± 0.37 %	3.50 % ± 1.17 %
M+2	1.90 % ± 0.16 %	5.02 % ± 0.19 %	5.83 % ± 0.32 %
M+3	2.79 % ± 0.23 %	6.45 % ± 0.65 %	23.07 % ± 1.53 %
M+4	1.62 % ± 0.16 %	3.86 % ± 0.21 %	6.32 % ± 1.63 %
M+5	1.87 % ± 0.16 %	3.49 % ± 0.40 %	3.17 % ± 0.93 %
M+6	35.94 % ± 1.15 %	32.14 % ± 1.31 %	22.05 % ± 1.33 %

9 mM [U-$^{13}C_6$]glucose *GP921*			
	Glucose in glycogen	Glucosamine	Muramic acid
M+1	1.88 % ± 0.57 %	3.00 % ± 0.52 %	4.17 % ± 1.01 %
M+2	2.20 % ± 0.23 %	4.15 % ± 0.46 %	5.64 % ± 1.17 %
M+3	3.84 % ± 0.36 %	6.29 % ± 0.49 %	22.15 % ± 1.58 %
M+4	1.63 % ± 0.29 %	2.98 % ± 0.33 %	6.82 % ± 0.98 %
M+5	2.01 % ± 0.40 %	3.68 % ± 0.35 %	2.04 % ± 0.53 %
M+6	33.98 % ± 1.51 %	31.80 % ± 1.04 %	21.13 % ± 1.10 %

9 mM [U-$^{13}C_6$]glucose *GP1562*

	Glucose in glycogen	Glucosamine	Muramic acid
M+1	1.62 % ± 0.61 %	3.90 % ± 2.47 %	6.66 % ± 1.87 %
M+2	1.98 % ± 0.60 %	4.73 % ± 2.66 %	6.66 % ± 3.18 %
M+3	3.38 % ± 1.59 %	4.93 % ± 2.38 %	19.11 % ± 2.39 %
M+4	1.43 % ± 0.94 %	4.28 % ± 2.48 %	9.60 % ± 1.83 %
M+5	3.01 % ± 1.15 %	3.13 % ± 1.73 %	2.37 % ± 1.44 %
M+6	34.20 % ± 1.60 %	29.67 % ± 2.11 %	20.76 % ± 1.61 %

Supplemental Table S64 Relative fractions of isotopologues (mol%) of amino acids from experiments with *NCIB3610*, *GP921* and *GP1562* grown on Msgg agar plate supplemented with 9 mM [U-$^{13}C_6$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from six independent experiments are shown.

9 mM [U-$^{13}C_6$]glucose *NCIB3610*								
	Ala	Asp	DAP	Glu	Gly	His	Ile	Leu
M+1	4.28 % ± 0.51 %	8.56 % ± 0.66 %	9.32 % ± 0.47 %	7.36 % ± 0.63 %	5.10 % ± 0.48 %	18.72 % ± 0.57 %	10.21 % ± 0.41 %	18.65 % ± 0.43 %
M+2	3.41 % ± 0.18 %	8.58 % ± 0.40 %	9.74 % ± 0.58 %	12.01 % ± 0.41 %	19.71 % ± 1.31 %	8.18 % ± 0.56 %	19.74 % ± 0.92 %	19.04 % ± 0.87 %
M+3	12.97 % ± 1.20 %	3.07 % ± 0.28 %	14.76 % ± 0.60 %	1.97 % ± 0.11 %		7.99 % ± 0.49 %	2.48 % ± 0.21 %	8.40 % ± 0.43 %
M+4		0.43 % ± 0.10 %	1.24 % ± 0.68 %	0.78 % ± 0.07 %		6.12 % ± 0.33 %	1.37 % ± 0.10 %	4.23 % ± 0.22 %

M+5			1.49 % ± 0.24 %	0.14 % ± 0.03 %		13.23 % ± 0.67 %	0.20 % ± 0.05 %	1.56 % ± 0.10 %
M+6			0.63 % ± 0.14 %			5.68 % ± 0.47 %		
M+7			0.15 % ± 0.12 %					

9 mM [U-$^{13}C_6$]glucose *NCIB3610*								
	Lys	Met	Phe	Pro	Ser	Thr	Tyr	Val
M+1	9.82 % ± 0.56 %	25.32 % ± 4.60 %	2.71 % ± 1.93 %	5.93 % ± 0.30 %	5.20 % ± 1.34 %	10.74 % ± 1.89 %	7.40 % ± 0.35 %	6.54 % ± 0.42 %
M+2	16.23 % ± 0.59 %	6.07 % ± 1.73 %	0.35 % ± 0.32 %	9.54 % ± 0.90 %	5.78 % ± 0.99 %	8.53 % ± 0.90 %	7.79 % ± 0.49 %	12.96 % ± 0.71 %
M+3	11.72 % ± 0.40 %	5.25 % ± 2.14 %	0.84 % ± 0.21 %	2.02 % ± 0.18 %	12.97 % ± 1.20 %	2.75 % ± 0.45 %	10.26 % ± 0.43 %	11.00 % ± 0.66 %
M+4	1.95 % ± 0.38 %	3.15 % ± 1.77 %	0.93 % ± 0.15 %	0.80 % ± 0.37 %		0.02 % ± 0.07 %	11.12 % ± 0.64 %	1.61 % ± 0.13 %
M+5	1.20 % ± 0.20 %	1.89 % ± 1.90 %	0.40 % ± 0.08 %	0.16 % ± 0.14 %			6.01 % ± 0.40 %	4.21 % ± 0.41 %
M+6	0.07 % ± 0.07 %		0.40 % ± 0.12 %				6.67 % ± 0.49 %	
M+7			0.27 % ± 0.10 %				4.36 % ± 0.13 %	
M+			0.06 ± 0.06				1.28 ± 0.17	

8			% %				% %	
M+9			0.05 % ± 0.04 %				1.84 % ± 0.19 %	

9 mM [U-$^{13}C_6$]glucose *GP921*								
	Ala	Asp	DAP	Glu	Gly	His	Ile	Leu
M+1	3.43 % ± 0.70 %	9.08 % ± 0.88 %	8.59 % ± 0.92 %	1.63 % ± 0.76 %	4.28 % ± 0.29 %	15.10 % ± 0.91 %	7.89 % ± 0.28 %	20.60 % ± 0.50 %
M+2	3.43 % ± 0.22 %	4.21 % ± 0.46 %	5.92 % ± 0.81 %	5.15 % ± 0.42 %	18.50 % ± 1.03 %	4.60 % ± 0.91 %	20.95 % ± 0.89 %	20.03 % ± 0.55 %
M+3	20.20 % ± 1.14 %	7.59 % ± 0.70 %	18.43 % ± 1.03 %	0.71 % ± 0.22 %		5.37 % ± 0.70 %	4.57 % ± 0.21 %	10.52 % ± 0.44 %
M+4		2.57 % ± 0.28 %	3.34 % ± 0.86 %	0.32 % ± 0.07 %		3.73 % ± 0.39 %	2.53 % ± 0.19 %	5.31 % ± 0.23 %
M+5			0.99 % ± 0.33 %	0.11 % ± 0.03 %		9.65 % ± 0.83 %	1.30 % ± 0.13 %	2.68 % ± 0.18 %
M+6			2.28 % ± 0.37 %			4.45 % ± 0.60 %		
M+7			0.89 % ± 0.21 %					

9 mM [U-$^{13}C_6$]glucose *GP921*								
	Lys	Met	Phe	Pro	Ser	Thr	Tyr	Val

M+1	8.12 %	±	0.87 %	18.25 %	±	7.10 %	1.92 %	±	1.72 %	1.22 %	±	0.43 %	6.03 %	±	1.52 %	12.20 %	±	2.95 %	5.24 %	±	0.91 %	4.80 %	±	0.52 %
M+2	13.81 %	±	0.61 %	7.17 %	±	5.09 %	0.04 %	±	0.13 %	2.36 %	±	0.44 %	6.27 %	±	0.59 %	3.57 %	±	1.33 %	7.25 %	±	0.64 %	16.16 %	±	0.41 %
M+3	13.97 %	±	0.92 %	4.94 %	±	4.22 %	0.17 %	±	0.20 %	0.86 %	±	0.14 %	12.47 %	±	0.90 %	7.83 %	±	0.93 %	9.07 %	±	0.66 %	14.20 %	±	0.47 %
M+4	2.51 %	±	0.44 %	3.19 %	±	3.10 %	0.22 %	±	0.09 %	0.48 %	±	0.45 %				1.87 %	±	0.76 %	7.28 %	±	0.64 %	1.24 %	±	0.10 %
M+5	3.07 %	±	0.27 %	4.74 %	±	5.56 %	0.11 %	±	0.10 %	0.07 %	±	0.11 %							5.56 %	±	0.55 %	7.06 %	±	0.65 %
M+6	1.12 %	±	0.19 %				0.09 %	±	0.14 %										5.97 %	±	0.57 %			
M+7							0.07 %	±	0.08 %										4.05 %	±	0.60 %			
M+8							0.02 %	±	0.03 %										1.00 %	±	0.40 %			
M+9							0.01 %	±	0.04 %										2.60 %	±	0.53 %			

9 mM [U-$^{13}C_6$]glucose *GP1562*																							
	Ala			Asp			DAP			Glu			Gly			His			Ile			Leu		
M+1	6.22 %	±	0.55 %	8.14 %	±	0.85 %	9.59 %	±	0.84 %	5.42 %	±	0.59 %	6.96 %	±	0.31 %	18.17 %	±	0.62 %	9.46 %	±	0.47 %	16.88 %	±	0.96 %
M+	5.13	±	0.20	10.57	±	0.60	11.18	±	0.67	12.17	±	0.31	32.97	±	0.64	10.75	±	0.55	28.53	±	0.96	25.48	±	1.40

2	% %	% %	% %	% %	% %	% %	% %	% %
M+3	24.59 % ± 0.90 %	4.80 % ± 0.28 %	21.79 % ± 1.05 %	2.36 % ± 0.19 %		11.84 % ± 0.49 %	4.31 % ± 0.40 %	14.04 % ± 0.94 %
M+4		1.17 % ± 0.17 %	2.63 % ± 1.08 %	1.31 % ± 0.10 %		8.00 % ± 0.40 %	2.81 % ± 0.16 %	7.03 % ± 0.47 %
M+5			3.10 % ± 0.38 %	0.31 % ± 0.04 %		18.60 % ± 1.15 %	0.57 % ± 0.13 %	3.14 % ± 0.20 %
M+6			1.26 % ± 0.15 %			9.01 % ± 0.39 %		
M+7			0.29 % ± 0.15 %					

9 mM [U-$^{13}C_6$]glucose *GP1562*								
	Lys	Met	Phe	Pro	Ser	Thr	Tyr	Val
M+1	9.47 % ± 0.68 %	28.17 % ± 9.39 %	1.79 % ± 1.42 %	5.42 % ± 0.65 %	8.92 % ± 1.10 %	10.07 % ± 1.43 %	7.85 % ± 0.47 %	6.94 % ± 0.43 %
M+2	18.99 % ± 0.75 %	11.20 % ± 3.35 %	0.06 % ± 0.14 %	10.57 % ± 0.70 %	9.43 % ± 0.61 %	9.94 % ± 0.92 %	10.41 % ± 0.41 %	19.89 % ± 0.81 %
M+3	14.68 % ± 0.60 %	5.62 % ± 6.07 %	0.31 % ± 0.13 %	2.75 % ± 0.21 %	24.50 % ± 1.05 %	5.10 % ± 0.62 %	14.41 % ± 0.61 %	17.81 % ± 0.82 %
M+4	3.23 % ± 0.45 %	6.23 % ± 3.07 %	0.44 % ± 0.15 %	1.33 % ± 0.53 %		0.51 % ± 0.27 %	15.59 % ± 0.50 %	2.81 % ± 0.15 %
M+5	1.89 % ± 0.20 %	1.27 % ± 1.45 %	0.21 % ± 0.09 %	0.34 % ± 0.21 %			9.26 % ± 0.43 %	8.00 % ± 0.41 %

M+6	0.28 % ± 0.15 %		0.13 % ± 0.07 %				10.18 % ± 0.39 %	
M+7			0.16 % ± 0.09 %				6.73 % ± 0.50 %	
M+8			0.02 % ± 0.04 %				1.93 % ± 0.22 %	
M+9			0.02 % ± 0.02 %				3.18 % ± 0.32 %	

Supplemental Table S65 Relative fractions of isotopologues (mol%) of polar metabolites from experiments with *NCIB3610*, *GP921* and *GP1562* grown on Msgg agar plate supplemented with 9 mM [U-$^{13}C_6$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from six independent experiments are shown.

9 mM [U-$^{13}C_6$]glucose *NCIB3610*								
	Lactate	Glycolic acid	Alanine	Glycine	Oxalic acid	Valine	leucine	Isoleucine
M+1	0.31 % ± 0.24 %	2.71 % ± 0.68 %	4.22 % ± 0.47 %	3.93 % ± 0.77 %	2.55 % ± 1.44 %	6.32 % ± 0.70 %	17.85 % ± 1.06 %	12.43 % ± 0.79 %
M+2	0.65 % ± 0.22 %	6.10 % ± 0.54 %	2.54 % ± 0.24 %	11.11 % ± 0.72 %	3.78 % ± 1.91 %	8.05 % ± 0.40 %	16.91 % ± 1.19 %	14.98 % ± 0.82 %
M+3	1.63 % ± 0.56 %		4.49 % ± 0.40 %			6.01 % ± 0.50 %	6.36 % ± 0.68 %	2.19 % ± 0.47 %
M+4						0.64 % ± 0.18 %	3.48 % ± 0.52 %	0.69 % ± 0.29 %
M+5						2.03 % ± 0.18 %	1.32 % ± 0.25 %	0.15 % ± 0.11 %

9 mM [U-$^{13}C_6$]glucose *NCIB3610*								
	Succinic acid	Fumaric acid	Glycerol	Methionine	Serine	Threonine	Phenylalanine	Malic acid
M+1	3.67 % ± 2.14 %	7.34 % ± 0.44 %	0.68 % ± 0.98 %	25.53 % ± 1.82 %	4.88 % ± 1.29 %	11.56 % ± 3.50 %	2.59 % ± 2.15 %	7.54 % ± 2.53 %
M+2	6.84 % ± 1.58 %	9.00 % ± 0.39 %	0.27 % ± 0.38 %	6.79 % ± 0.98 %	4.45 % ± 0.67 %	7.08 % ± 2.79 %	0.79 % ± 0.94 %	7.23 % ± 3.56 %
M+3	1.13 % ± 0.25 %	2.25 % ± 0.20 %	0.72 % ± 0.59 %	4.27 % ± 0.91 %	3.57 % ± 0.82 %	5.32 % ± 5.82 %	1.59 % ± 0.82 %	1.32 % ± 1.08 %
M+4	0.12 % ± 0.05 %	0.33 % ± 0.10 %		3.34 % ± 0.60 %		2.10 % ± 3.21 %	1.44 % ± 0.51 %	0.19 % ± 0.28 %
M+5				1.20 % ± 0.29 %			0.73 % ± 0.54 %	
M+6							0.60 % ± 0.37 %	
M+7							0.00 % ± 0.00 %	
M+8							0.00 % ± 0.00 %	
M+9							0.00 % ± 0.00 %	

9 mM [U-$^{13}C_6$]glucose *NCIB3610*

	Aspartate	Glutamate	Oelic acid	Stearic acid	Citric acid
M+1	8.47 % ± 1.66 %	8.13 % ± 0.84 %	1.45 % ± 1.50 %	0.67 % ± 0.29 %	
M+2	8.23 % ± 0.86 %	12.05 % ± 0.79 %	3.39 % ± 2.48 %	0.12 % ± 0.08 %	
M+3	2.22 % ± 0.58 %	1.83 % ± 0.41 %	1.30 % ± 1.42 %	0.07 % ± 0.03 %	
M+4	0.17 % ± 0.23 %	0.71 % ± 0.18 %	0.97 % ± 1.26 %	0.16 % ± 0.04 %	
M+5		0.15 % ± 0.14 %	0.82 % ± 0.88 %	0.05 % ± 0.01 %	
M+6			0.32 % ± 0.77 %	0.10 % ± 0.03 %	
M+7			0.41 % ± 0.82 %	0.03 % ± 0.01 %	
M+8			0.16 % ± 0.25 %	0.06 % ± 0.02 %	
M+9			0.09 % ± 0.22 %	0.01 % ± 0.01 %	
M+10			0.15 % ± 0.26 %	0.02 % ± 0.01 %	
M+11			0.03 % ± 0.07 %	0.01 % ± 0.01 %	
M+12			0.18 % ± 0.26 %	0.00 % ± 0.01 %	
M+13			0.22 % ± 0.39 %	0.00 % ± 0.00 %	
M+14			0.09 % ± 0.12 %	0.00 % ± 0.00 %	
M+15			0.08 % ± 0.15 %	0.01 % ± 0.01 %	
M+16			0.45 % ± 0.32 %	0.01 % ± 0.01 %	
M+17			0.10 % ± 0.23 %	0.00 % ± 0.00 %	

M+18			0.13 % ± 0.18 %	0.00 % ± 0.00 %	

9 mM [U-$^{13}C_6$]glucose *GP921*								
	Lactate	Glycolic acid	Alanine	Glycine	Oxalic acid	Valine	leucine	Isoleucine
M+1	0.17 % ± 0.35 %	2.18 % ± 1.47 %	4.18 % ± 1.22 %	1.80 % ± 1.88 %	0.51 % ± 0.74 %	7.39 % ± 1.05 %	20.32 % ± 2.95 %	11.03 % ± 3.37 %
M+2	0.40 % ± 0.46 %	3.50 % ± 1.54 %	3.14 % ± 0.40 %	8.16 % ± 1.01 %	5.65 % ± 6.16 %	13.06 % ± 0.93 %	17.74 % ± 2.13 %	16.20 % ± 3.48 %
M+3	1.32 % ± 0.99 %		12.47 % ± 4.66 %			10.19 % ± 0.79 %	8.07 % ± 1.75 %	3.35 % ± 1.83 %
M+4						0.92 % ± 0.36 %	3.59 % ± 1.42 %	1.61 % ± 1.23 %
M+5						4.11 % ± 0.45 %	1.81 % ± 0.99 %	0.35 % ± 0.57 %

9 mM [U-$^{13}C_6$]glucose *GP921*								
	Succinic acid	Fumaric acid	Glycerol	Methionine	Serine	Threonine	Phenylalanine	Malic acid
M+1	0.01 % ± 0.04 %	4.39 % ± 0.86 %	2.09 % ± 2.14 %	20.26 % ± 2.33 %	6.10 % ± 2.60 %	19.96 % ± 4.70 %	3.70 % ± 1.88 %	5.92 % ± 7.29 %
M+2	4.11 % ± 0.72 %	4.59 % ± 0.59 %	0.28 % ± 0.30 %	4.10 % ± 2.29 %	5.74 % ± 2.25 %	1.71 % ± 1.97 %	1.10 % ± 0.93 %	4.16 % ± 4.66 %
M+3	0.67 % ± 0.17 %	4.72 % ± 0.66 %	0.26 % ± 0.42 %	4.91 % ± 2.27 %	3.33 % ± 1.87 %	21.80 % ± 3.17 %	2.24 % ± 0.90 %	2.99 % ± 3.65 %

M+4	0.26 % ± 0.05 %	1.62 % ± 0.32 %		4.90 % ± 1.13 %		10.01 % ± 2.50 %	1.75 % ± 0.85 %	1.78 % ± 2.39 %
M+5				1.46 % ± 1.06 %			1.26 % ± 1.02 %	
M+6							0.73 % ± 0.65 %	
M+7							0.69 % ± 0.64 %	
M+8							0.00 % ± 0.00 %	
M+9							0.04 % ± 0.09 %	

9 mM [U-$^{13}C_6$]glucose *GP921*					
	Aspartate	Glutamate	Oelic acid	Stearic acid	Citric acid
M+1	5.98 % ± 8.32 %	2.61 % ± 1.88 %	1.15 % ± 2.62 %	0.39 % ± 0.38 %	5.98 % ± 2.35 %
M+2	4.97 % ± 4.56 %	6.46 % ± 1.31 %	3.08 % ± 1.77 %	0.05 % ± 0.07 %	8.29 % ± 2.78 %
M+3	3.82 % ± 4.59 %	0.85 % ± 0.62 %	0.68 % ± 0.92 %	0.03 % ± 0.03 %	1.31 % ± 0.94 %
M+4	0.43 % ± 0.65 %	0.39 % ± 0.32 %	0.22 % ± 0.49 %	0.12 % ± 0.04 %	1.36 % ± 1.38 %
M+5		0.14 % ± 0.09 %	0.72 % ± 1.68 %	0.03 % ± 0.01 %	0.44 % ± 0.67 %
M+6			0.30 % ± 0.55 %	0.12 % ± 0.05 %	0.01 % ± 0.03 %
M+7			0.17 % ± 0.33 %	0.02 % ± 0.01 %	

M+8			0.52 % ± 1.11 %	0.09 % ± 0.04 %	
M+9			0.12 % ± 0.23 %	0.01 % ± 0.00 %	
M+10			0.10 % ± 0.29 %	0.04 % ± 0.02 %	
M+11			0.19 % ± 0.40 %	0.01 % ± 0.01 %	
M+12			0.22 % ± 0.56 %	0.01 % ± 0.02 %	
M+13			0.36 % ± 0.69 %	0.01 % ± 0.00 %	
M+14			0.07 % ± 0.16 %	0.00 % ± 0.00 %	
M+15			0.21 % ± 0.36 %	0.00 % ± 0.01 %	
M+16			0.70 % ± 0.97 %	0.01 % ± 0.00 %	
M+17			0.34 % ± 0.69 %	0.00 % ± 0.00 %	
M+18			0.31 % ± 0.89 %	0.00 % ± 0.00 %	

9 mM [U-$^{13}C_6$]glucose *GP1562*								
	Lactate	Glycolic acid	Alanine	Glycine	Oxalic acid	Valine	leucine	Isoleucine
M+1	0.01 % ± 0.03 %	1.86 % ± 1.40 %	5.42 % ± 2.49 %	2.98 % ± 2.71 %	3.47 % ± 4.38 %	7.62 % ± 1.76 %	16.40 % ± 5.11 %	12.92 % ± 5.67 %
M+2	0.16 % ± 0.20 %	6.60 % ± 1.88 %	3.05 % ± 2.12 %	24.53 % ± 2.92 %	6.80 % ± 6.73 %	15.13 % ± 3.21 %	21.78 % ± 5.85 %	18.37 % ± 4.89 %
M+3	0.76 % ± 0.30 %		11.65 % ± 3.80 %			12.26 % ± 3.55 %	10.78 % ± 4.42 %	3.74 % ± 2.04 %

M+4						1.78 % ± 1.05 %	6.55 % ± 4.17 %	2.57 % ± 2.51 %
M+5						4.65 % ± 2.33 %	2.87 % ± 2.15 %	1.20 % ± 2.04 %

9 mM [U-$^{13}C_6$]glucose *GP1562*								
	Succinic acid	Fumaric acid	Glycerol	Methionine	Serine	Threonine	Phenylalanine	Malic acid
M+1	3.17 % ± 4.40 %	4.99 % ± 1.11 %	2.55 % ± 2.61 %	29.53 % ± 8.11 %	6.14 % ± 3.45 %	13.98 % ± 12.05 %	2.03 % ± 2.96 %	15.78 % ± 27.02 %
M+2	10.53 % ± 8.06 %	10.03 % ± 0.96 %	0.28 % ± 0.41 %	7.94 % ± 7.73 %	4.30 % ± 2.61 %	6.04 % ± 7.56 %	0.60 % ± 1.14 %	16.81 % ± 27.15 %
M+3	2.28 % ± 2.50 %	3.33 % ± 0.73 %	0.74 % ± 0.54 %	7.61 % ± 6.25 %	10.47 % ± 3.76 %	9.10 % ± 10.17 %	0.76 % ± 1.20 %	1.19 % ± 2.36 %
M+4	0.36 % ± 0.59 %	0.84 % ± 0.44 %		5.85 % ± 4.19 %		5.96 % ± 6.39 %	0.59 % ± 0.68 %	13.45 % ± 15.03 %
M+5				4.69 % ± 5.34 %			0.80 % ± 0.99 %	
M+6							1.05 % ± 1.56 %	
M+7							0.27 % ± 0.52 %	
M+8							0.69 % ± 1.28 %	
M+							0.34 ± 0.80	

9							% %	

9 mM [U-$^{13}C_6$]glucose *GP1562*					
	Aspartate	Glutamate	Oelic acid	Stearic acid	Citric acid
M+1	6.17 % ± 5.27 %	5.82 % ± 1.18 %	1.90 % ± 3.48 %	0.51 % ± 0.27 %	12.90 % ± 9.26 %
M+2	11.72 % ± 8.90 %	12.66 % ± 0.68 %	3.18 % ± 2.92 %	0.05 % ± 0.06 %	12.25 % ± 8.76 %
M+3	2.60 % ± 2.38 %	2.15 % ± 0.34 %	0.77 % ± 1.64 %	0.03 % ± 0.03 %	7.54 % ± 10.55 %
M+4	0.61 % ± 0.76 %	1.17 % ± 0.27 %	0.61 % ± 1.19 %	0.04 % ± 0.02 %	8.54 % ± 14.30 %
M+5		0.21 % ± 0.12 %	0.55 % ± 1.17 %	0.02 % ± 0.01 %	3.49 % ± 10.30 %
M+6			1.59 % ± 2.97 %	0.03 % ± 0.01 %	5.39 % ± 13.32 %
M+7			0.81 % ± 2.30 %	0.01 % ± 0.01 %	
M+8			0.80 % ± 1.57 %	0.02 % ± 0.01 %	
M+9			0.65 % ± 1.64 %	0.00 % ± 0.00 %	
M+10			0.21 % ± 0.31 %	0.01 % ± 0.01 %	
M+11			0.79 % ± 1.38 %	0.01 % ± 0.01 %	
M+12			0.27 % ± 0.81 %	0.00 % ± 0.01 %	
M+13			0.61 % ± 1.33 %	0.00 % ± 0.00 %	
M+14			0.27 % ± 0.40 %	0.00 % ± 0.00 %	
M+15			0.48 % ± 0.59 %	0.01 % ± 0.01 %	

M+16			0.62 % ± 1.18 %	0.01 % ± 0.01 %	
M+17			0.97 % ± 1.91 %	0.00 % ± 0.00 %	
M+18			0.72 % ± 1.57 %	0.00 % ± 0.00 %	

Supplemental Table S66 Relative fractions of isotopologues (mol%) of sugars from experiments with *NCIB3610*, *GP921* and *GP1562* grown on Msgg agar plate supplemented with 9 mM [U-$^{13}C_6$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from six independent experiments are shown.

9 mM [U-$^{13}C_6$]glucose *NCIB3610*			
	Glucose in glycogen	Glucosamine	Muramic acid
M+1	4.27 % ± 2.31 %	7.25 % ± 0.83 %	7.37 % ± 3.15 %
M+2	4.01 % ± 0.89 %	5.85 % ± 0.62 %	6.27 % ± 2.67 %
M+3	3.20 % ± 0.79 %	4.31 % ± 1.28 %	3.92 % ± 2.97 %
M+4	1.51 % ± 0.95 %	2.88 % ± 0.51 %	4.62 % ± 2.01 %
M+5	1.84 % ± 1.49 %	3.27 % ± 0.64 %	1.35 % ± 0.95 %
M+6	10.35 % ± 3.97 %	9.63 % ± 1.57 %	5.96 % ± 1.71 %

9 mM [U-$^{13}C_6$]glucose *GP921*			
	Glucose in glycogen	Glucosamine	Muramic acid
M+1	2.93 % ± 2.04 %	5.87 % ± 6.26 %	5.23 % ± 6.22 %

M+2	1.76 % ± 1.59 %	4.50 % ± 4.36 %	6.51 % ± 5.74 %
M+3	3.13 % ± 1.59 %	3.73 % ± 3.15 %	8.01 % ± 7.57 %
M+4	0.80 % ± 0.72 %	2.36 % ± 3.39 %	4.05 % ± 5.13 %
M+5	1.00 % ± 0.67 %	2.95 % ± 3.34 %	2.29 % ± 3.78 %
M+6	12.84 % ± 2.54 %	10.77 % ± 4.37 %	8.96 % ± 5.10 %

9 mM [U-$^{13}C_6$]glucose *GP1562*			
	Glucose in glycogen	Glucosamine	Muramic acid
M+1	5.64 % ± 4.45 %	6.32 % ± 3.31 %	8.79 % ± 5.45 %
M+2	5.24 % ± 2.04 %	8.74 % ± 3.63 %	11.09 % ± 6.01 %
M+3	5.35 % ± 3.42 %	7.50 % ± 4.36 %	9.98 % ± 6.58 %
M+4	4.20 % ± 3.23 %	4.82 % ± 2.81 %	12.51 % ± 5.82 %
M+5	2.71 % ± 3.70 %	5.93 % ± 3.05 %	3.00 % ± 2.32 %
M+6	24.25 % ± 8.40 %	23.91 % ± 6.03 %	15.39 % ± 6.00 %

Supplemental Table S67 Relative fractions of isotopologues (mol%) of amino acids from experiments with *NCIB3610*, *GP921* and *GP1562* grown on Msgg agar plate supplemented with 6 mM [U-$^{13}C_5$]glutamate. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

6 mM [U-$^{13}C_5$]glutamate *NCIB3610*

	Ala	Asp	DAP	Glu	Gly	His	Ile	Leu
M+1	3.27 % ± 0.27 %	6.59 % ± 0.30 %	6.24 % ± 0.39 %	5.81 % ± 0.25 %	1.14 % ± 0.09 %	2.29 % ± 0.84 %	8.12 % ± 0.38 %	9.44 % ± 0.37 %
M+2	1.35 % ± 0.12 %	4.61 % ± 0.18 %	5.69 % ± 0.26 %	3.65 % ± 0.20 %	2.20 % ± 0.06 %	0.00 % ± 0.00 %	6.17 % ± 0.16 %	7.00 % ± 0.17 %
M+3	3.16 % ± 0.09 %	0.91 % ± 0.04 %	3.63 % ± 0.38 %	3.30 % ± 0.10 %		0.14 % ± 0.13 %	8.89 % ± 0.15 %	0.60 % ± 0.06 %
M+4		7.27 % ± 0.25 %	7.82 % ± 0.25 %	0.33 % ± 0.03 %		0.28 % ± 0.55 %	0.35 % ± 0.03 %	0.17 % ± 0.01 %
M+5			0.33 % ± 0.23 %	8.56 % ± 0.37 %		0.25 % ± 0.45 %	0.30 % ± 0.02 %	0.01 % ± 0.01 %
M+6			0.09 % ± 0.08 %			0.03 % ± 0.04 %		
M+7			0.27 % ± 0.06 %					

6 mM [U-$^{13}C_5$]glutamate *NCIB3610*								
	Lys	Met	Phe	Pro	Ser	Thr	Tyr	Val
M+1	6.35 % ± 0.59 %	5.54 % ± 0.38 %	2.13 % ± 0.31 %	4.30 % ± 0.14 %	1.83 % ± 0.30 %	7.33 % ± 0.32 %	2.82 % ± 0.37 %	4.98 % ± 0.26 %
M+2	5.88 % ± 0.52 %	4.47 % ± 0.25 %	0.91 % ± 0.10 %	1.81 % ± 0.18 %	1.06 % ± 0.14 %	5.10 % ± 0.20 %	2.80 % ± 0.22 %	4.25 % ± 0.24 %
M+3	7.13 % ± 0.20 %	0.67 % ± 0.12 %	0.86 % ± 0.02 %	3.46 % ± 0.11 %	1.03 % ± 0.06 %	0.83 % ± 0.05 %	2.04 % ± 0.08 %	3.29 % ± 0.10 %

M+4	4.69 % ± 0.44 %	8.19 % ± 0.18 %	0.15 % ± 0.02 %	0.57 % ± 0.09 %		7.98 % ± 0.12 %	0.00 % ± 0.01 %	0.17 % ± 0.02 %
M+5	0.25 % ± 0.32 %	0.07 % ± 0.05 %	0.06 % ± 0.04 %	9.41 % ± 0.52 %			0.12 % ± 0.10 %	0.13 % ± 0.01 %
M+6	0.27 % ± 0.10 %		0.00 % ± 0.00 %				0.00 % ± 0.00 %	
M+7			0.00 % ± 0.01 %				0.00 % ± 0.00 %	
M+8			0.00 % ± 0.00 %				0.01 % ± 0.02 %	
M+9			0.00 % ± 0.00 %				0.00 % ± 0.00 %	

6 mM [U-$^{13}C_5$]glutamate *GP921*								
	Ala	Asp	DAP	Glu	Gly	His	Ile	Leu
M+1	2.62 % ± 0.28 %	5.32 % ± 0.48 %	5.60 % ± 0.16 %	2.95 % ± 0.30 %	0.66 % ± 0.19 %	0.02 % ± 0.04 %	7.23 % ± 0.42 %	9.27 % ± 0.22 %
M+2	1.43 % ± 0.06 %	3.75 % ± 0.12 %	4.78 % ± 0.13 %	2.87 % ± 0.15 %	1.48 % ± 0.07 %	0.00 % ± 0.00 %	7.25 % ± 0.08 %	8.66 % ± 0.07 %
M+3	5.01 % ± 0.05 %	1.84 % ± 0.10 %	5.91 % ± 0.15 %	3.18 % ± 0.09 %		0.03 % ± 0.10 %	8.79 % ± 0.10 %	0.77 % ± 0.04 %
M+4		8.86 % ± 0.11 %	8.49 % ± 0.09 %	0.47 % ± 0.02 %		0.04 % ± 0.08 %	0.38 % ± 0.04 %	0.25 % ± 0.02 %
M+			0.39 ± 0.05	12.86 ± 0.21		0.06 ± 0.04	0.39 ± 0.01	0.01 ± 0.01

5			% %	% %		% %	% %	% %	
M+6			0.19 % ± 0.03 %			0.05 % ± 0.04 %			
M+7			0.41 % ± 0.04 %						

6 mM [U-$^{13}C_5$]glutamate *GP921*								
	Lys	Met	Phe	Pro	Ser	Thr	Tyr	Val
M+1	5.18 % ± 0.26 %	4.71 % ± 0.33 %	0.45 % ± 0.37 %	2.26 % ± 0.17 %	0.49 % ± 0.30 %	6.74 % ± 0.41 %	1.08 % ± 0.38 %	4.43 % ± 0.29 %
M+2	4.60 % ± 0.19 %	2.77 % ± 0.27 %	0.00 % ± 0.00 %	1.18 % ± 0.08 %	0.42 % ± 0.12 %	3.98 % ± 0.25 %	1.97 % ± 0.20 %	5.66 % ± 0.09 %
M+3	7.31 % ± 0.13 %	1.46 % ± 0.19 %	0.00 % ± 0.00 %	2.82 % ± 0.06 %	0.95 % ± 0.03 %	1.72 % ± 0.05 %	1.37 % ± 0.10 %	4.53 % ± 0.07 %
M+4	4.21 % ± 0.06 %	7.23 % ± 0.61 %	0.12 % ± 0.03 %	0.64 % ± 0.06 %		8.32 % ± 0.07 %	0.00 % ± 0.00 %	0.22 % ± 0.02 %
M+5	0.06 % ± 0.03 %	0.36 % ± 0.36 %	0.02 % ± 0.02 %	12.15 % ± 0.21 %			0.14 % ± 0.05 %	0.24 % ± 0.01 %
M+6	0.23 % ± 0.03 %		0.00 % ± 0.00 %				0.00 % ± 0.00 %	
M+7			0.00 % ± 0.00 %				0.00 % ± 0.00 %	
M+8			0.02 % ± 0.02 %				0.02 % ± 0.02 %	

M+9			0.00 % ± 0.00 %				0.01 % ± 0.04 %	

6 mM [U-$^{13}C_5$]glutamate *GP1562*								
	Ala	Asp	DAP	Glu	Gly	His	Ile	Leu
M+1	2.94 % ± 0.30 %	5.95 % ± 0.33 %	6.58 % ± 0.31 %	3.95 % ± 0.24 %	0.79 % ± 0.12 %	1.71 % ± 0.29 %	8.12 % ± 0.24 %	10.47 % ± 0.21 %
M+2	1.58 % ± 0.06 %	5.44 % ± 0.13 %	6.57 % ± 0.24 %	3.06 % ± 0.20 %	2.38 % ± 0.03 %	0.00 % ± 0.00 %	6.69 % ± 0.16 %	8.21 % ± 0.14 %
M+3	4.35 % ± 0.04 %	0.91 % ± 0.04 %	4.51 % ± 0.14 %	3.78 % ± 0.07 %		0.25 % ± 0.10 %	9.85 % ± 0.17 %	0.83 % ± 0.05 %
M+4		9.62 % ± 0.13 %	9.11 % ± 0.13 %	0.49 % ± 0.02 %		0.02 % ± 0.04 %	0.46 % ± 0.05 %	0.23 % ± 0.01 %
M+5			0.46 % ± 0.06 %	11.89 % ± 0.23 %		0.07 % ± 0.04 %	0.40 % ± 0.01 %	0.01 % ± 0.01 %
M+6			0.20 % ± 0.04 %			0.09 % ± 0.06 %		
M+7			0.37 % ± 0.05 %					

6 mM [U-$^{13}C_5$]glutamate *GP1562*								
	Lys	Met	Phe	Pro	Ser	Thr	Tyr	Val
M+	6.76 ± 0.21	5.22 ± 0.26	1.21 ± 0.19	4.07 % ± 0.16	1.02 ± 0.44	6.56 ± 0.36	3.73 ± 0.45	4.97 ± 0.28

1	% %	% %	% %	%	% %	% %	% %	% %
M+2	6.13 % ± 0.13 %	4.63 % ± 0.19 %	0.29 % ± 0.16 %	1.83 % ± 0.09 %	0.83 % ± 0.14 %	5.59 % ± 0.17 %	3.65 % ± 0.16 %	5.01 % ± 0.18 %
M+3	7.83 % ± 0.15 %	0.73 % ± 0.12 %	0.29 % ± 0.08 %	3.78 % ± 0.07 %	1.54 % ± 0.03 %	0.80 % ± 0.08 %	2.90 % ± 0.10 %	4.04 % ± 0.06 %
M+4	5.01 % ± 0.09 %	8.79 % ± 0.16 %	0.15 % ± 0.03 %	0.62 % ± 0.07 %		9.53 % ± 0.12 %	0.07 % ± 0.05 %	0.19 % ± 0.02 %
M+5	0.16 % ± 0.04 %	0.16 % ± 0.06 %	0.01 % ± 0.01 %	10.15 % ± 0.18 %			0.26 % ± 0.02 %	0.19 % ± 0.02 %
M+6	0.37 % ± 0.03 %		0.00 % ± 0.00 %				0.01 % ± 0.02 %	
M+7			0.00 % ± 0.00 %				0.00 % ± 0.00 %	
M+8			0.00 % ± 0.00 %				0.00 % ± 0.00 %	
M+9			0.00 % ± 0.00 %				0.00 % ± 0.00 %	

Supplemental Table S68 Relative fractions of isotopologues (mol%) of polar metabolites from experiments with *NCIB3610*, *GP921* and *GP1562* grown on Msgg agar plate supplemented with 6 mM [U-$^{13}C_5$]glutamate. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

6 mM [U-$^{13}C_5$]glutamate *NCIB3610*								
	Lactate	Glycolic acid	Alanine	Glycine	Valine	leucine	Isoleucine	Succinic acid

M+1	1.09 % ± 0.38 %	0.42 % ± 0.27 %	4.66 % ± 0.37 %	1.58 % ± 0.28 %	4.72 % ± 0.23 %	8.60 % ± 0.39 %	9.24 % ± 0.43 %	2.55 % ± 0.54 %
M+2	0.72 % ± 0.14 %	0.27 % ± 0.16 %	1.68 % ± 0.17 %	2.85 % ± 0.17 %	4.06 % ± 0.23 %	6.53 % ± 0.23 %	6.03 % ± 0.23 %	0.93 % ± 1.09 %
M+3	2.00 % ± 0.29 %		3.26 % ± 0.20 %		3.05 % ± 0.12 %	0.59 % ± 0.07 %	8.41 % ± 0.17 %	0.86 % ± 0.05 %
M+4					0.14 % ± 0.03 %	0.16 % ± 0.01 %	0.48 % ± 0.06 %	13.19 % ± 0.73 %
M+5					0.12 % ± 0.01 %	0.02 % ± 0.01 %	0.27 % ± 0.03 %	

6 mM [U-$^{13}C_5$]glutamate *NCIB3610*								
	Fumaric acid	Glycerol	Methionine	Serine	Threonine	Phenylalanine	Malic acid	Aspartate
M+1	4.05 % ± 0.38 %	0.09 % ± 0.20 %	5.25 % ± 0.30 %	5.90 % ± 0.34 %	4.31 % ± 0.64 %	1.73 % ± 0.29 %	6.72 % ± 1.00 %	7.34 % ± 0.40 %
M+2	4.19 % ± 0.15 %	0.01 % ± 0.03 %	3.31 % ± 0.15 %	3.13 % ± 0.26 %	4.91 % ± 0.15 %	1.43 % ± 0.14 %	2.85 % ± 1.14 %	3.90 % ± 0.21 %
M+3	1.04 % ± 0.06 %	0.51 % ± 0.13 %	0.73 % ± 0.05 %	0.71 % ± 0.09 %	0.80 % ± 0.10 %	0.82 % ± 0.08 %	2.75 % ± 0.43 %	1.17 % ± 0.08 %
M+4	7.58 % ± 0.32 %		6.46 % ± 0.22 %		8.29 % ± 0.15 %	0.23 % ± 0.03 %	7.11 % ± 0.72 %	6.22 % ± 0.24 %
M+5			0.14 % ± 0.04 %			0.21 % ± 0.09 %		
M+						0.13 ± 0.08		

6						% %		
M+7						0.00 % ± 0.00 %		
M+8						0.00 % ± 0.00 %		
M+9						0.00 % ± 0.00 %		

6 mM [U-$^{13}C_5$]glutamate *NCIB3610*					
	Glutamate	Palmitate	Oelic acid	Stearic acid	Citric acid
M+1	7.70 % ± 0.42 %	1.60 % ± 0.76 %	1.16 % ± 0.45 %	2.21 % ± 0.65 %	
M+2	3.83 % ± 0.25 %	6.02 % ± 0.42 %	10.19 % ± 0.79 %	4.78 % ± 1.85 %	
M+3	2.46 % ± 0.13 %	1.02 % ± 0.10 %	1.16 % ± 0.32 %	1.52 % ± 0.33 %	
M+4	0.28 % ± 0.02 %	1.49 % ± 0.57 %	4.87 % ± 0.24 %	3.36 % ± 1.95 %	
M+5	5.74 % ± 0.32 %	0.17 % ± 0.05 %	0.89 % ± 0.21 %	0.48 % ± 0.21 %	
M+6		0.50 % ± 0.42 %	2.53 % ± 0.17 %	2.00 % ± 1.51 %	
M+7		0.03 % ± 0.04 %	0.39 % ± 0.04 %	0.24 % ± 0.16 %	
M+8		0.17 % ± 0.19 %	1.03 % ± 0.14 %	1.03 % ± 0.83 %	
M+9		0.01 % ± 0.01 %	0.13 % ± 0.04 %	0.13 % ± 0.06 %	
M+10		0.04 % ± 0.06 %	0.33 % ± 0.05 %	0.41 % ± 0.32 %	

M+11		0.01 % ± 0.01 %	0.01 % ± 0.01 %	0.57 % ± 0.57 %	
M+12		0.01 % ± 0.01 %	0.13 % ± 0.06 %	0.28 % ± 0.19 %	
M+13		0.00 % ± 0.00 %	0.04 % ± 0.05 %	0.11 % ± 0.11 %	
M+14		0.00 % ± 0.00 %	0.05 % ± 0.04 %	0.07 % ± 0.05 %	
M+15		0.00 % ± 0.00 %	0.01 % ± 0.03 %	0.16 % ± 0.14 %	
M+16		0.01 % ± 0.03 %	0.03 % ± 0.04 %	0.08 % ± 0.05 %	
M+17			0.05 % ± 0.07 %	0.03 % ± 0.02 %	
M+18			0.00 % ± 0.00 %	0.08 % ± 0.04 %	

6 mM [U-$^{13}C_5$]glutamate *GP921*								
	Lactate	Glycolic acid	Alanine	Glycine	Valine	leucine	Isoleucine	Succinic acid
M+1	0.12 % ± 0.15 %	0.15 % ± 0.17 %	2.37 % ± 0.32 %	0.10 % ± 0.14 %	4.87 % ± 0.24 %	9.86 % ± 0.31 %	8.77 % ± 0.37 %	0.29 % ± 0.35 %
M+2	0.58 % ± 0.10 %	0.21 % ± 0.13 %	1.35 % ± 0.12 %	1.45 % ± 0.13 %	6.16 % ± 0.14 %	9.42 % ± 0.26 %	6.99 % ± 0.15 %	0.82 % ± 0.56 %
M+3	2.30 % ± 0.10 %		4.27 % ± 0.13 %		4.79 % ± 0.12 %	0.89 % ± 0.05 %	8.96 % ± 0.44 %	0.92 % ± 0.01 %
M+4					0.24 % ± 0.02 %	0.29 % ± 0.05 %	0.52 % ± 0.08 %	16.08 % ± 0.62 %
M+5					0.29 % ± 0.02 %	0.02 % ± 0.01 %	0.43 % ± 0.02 %	

6 mM [U-$^{13}C_5$]glutamate *GP921*								
	Fumaric acid	Glycerol	Methionine	Serine	Threonine	Phenylalanine	Malic acid	Aspartate
M+1	2.34 % ± 0.25 %	0.00 % ± 0.00 %	4.36 % ± 0.41 %	2.17 % ± 0.34 %	3.68 % ± 0.75 %	0.00 % ± 0.00 %	4.83 % ± 0.35 %	4.35 % ± 0.27 %
M+2	3.83 % ± 0.06 %	0.00 % ± 0.00 %	2.27 % ± 0.20 %	1.54 % ± 0.26 %	3.74 % ± 0.53 %	0.16 % ± 0.11 %	2.77 % ± 0.22 %	4.26 % ± 0.19 %
M+3	1.61 % ± 0.03 %	0.18 % ± 0.18 %	1.21 % ± 0.12 %	0.23 % ± 0.10 %	1.51 % ± 0.18 %	0.09 % ± 0.06 %	3.79 % ± 0.12 %	1.49 % ± 0.10 %
M+4	9.85 % ± 0.25 %		6.89 % ± 0.22 %		8.97 % ± 0.45 %	0.16 % ± 0.05 %	9.33 % ± 0.30 %	10.43 % ± 0.32 %
M+5			0.07 % ± 0.04 %			0.32 % ± 0.08 %		
M+6						0.04 % ± 0.05 %		
M+7						0.00 % ± 0.00 %		
M+8						0.00 % ± 0.00 %		
M+9						0.00 % ± 0.00 %		

6 mM [U-$^{13}C_5$]glutamate *GP921*

	Glutamate	Palmitate	Oelic acid	Stearic acid	Citric acid
M+1	2.87 % ± 0.59 %	0.00 % ± 0.00 %	1.20 % ± 0.53 %	0.91 % ± 0.37 %	7.55 % ± 0.49 %
M+2	2.74 % ± 0.22 %	2.99 % ± 0.44 %	10.19 % ± 0.75 %	1.40 % ± 0.39 %	7.89 % ± 0.25 %
M+3	3.17 % ± 0.10 %	0.45 % ± 0.04 %	1.34 % ± 0.25 %	0.38 % ± 0.10 %	3.21 % ± 0.16 %
M+4	0.48 % ± 0.03 %	0.51 % ± 0.08 %	4.26 % ± 0.27 %	0.68 % ± 0.19 %	8.34 % ± 0.42 %
M+5	11.45 % ± 0.19 %	0.06 % ± 0.01 %	0.97 % ± 0.24 %	0.09 % ± 0.03 %	2.37 % ± 0.34 %
M+6		0.06 % ± 0.03 %	2.10 % ± 0.17 %	0.29 % ± 0.10 %	0.73 % ± 0.10 %
M+7		0.00 % ± 0.00 %	0.37 % ± 0.06 %	0.03 % ± 0.01 %	
M+8		0.00 % ± 0.00 %	0.77 % ± 0.10 %	0.12 % ± 0.04 %	
M+9		0.00 % ± 0.00 %	0.18 % ± 0.09 %	0.02 % ± 0.00 %	
M+10		0.00 % ± 0.00 %	0.25 % ± 0.04 %	0.06 % ± 0.02 %	
M+11		0.00 % ± 0.00 %	0.03 % ± 0.03 %	0.06 % ± 0.11 %	
M+12		0.00 % ± 0.00 %	0.08 % ± 0.06 %	0.03 % ± 0.03 %	
M+13		0.00 % ± 0.00 %	0.03 % ± 0.03 %	0.01 % ± 0.02 %	
M+14		0.00 % ± 0.00 %	0.10 % ± 0.06 %	0.03 % ± 0.02 %	
M+15		0.00 % ± 0.00 %	0.01 % ± 0.03 %	0.04 % ± 0.04 %	
M+16		0.00 % ± 0.00 %	0.04 % ± 0.04 %	0.02 % ± 0.01 %	
M+17			0.01 % ± 0.01 %	0.00 % ± 0.00 %	

M+18			0.12 % ± 0.10 %	0.01 % ± 0.01 %	

6 mM [U-$^{13}C_5$]glutamate *GP1562*								
	Lactate	Glycolic acid	Alanine	Glycine	Valine	leucine	Isoleucine	Succinic acid
M+1	0.00 % ± 0.00 %	0.08 % ± 0.11 %	1.70 % ± 0.27 %	0.00 % ± 0.00 %	3.80 % ± 0.44 %	9.02 % ± 0.77 %	9.30 % ± 0.35 %	22.00 % ± 20.16 %
M+2	0.01 % ± 0.02 %	0.27 % ± 0.22 %	0.85 % ± 0.13 %	1.44 % ± 0.26 %	4.14 % ± 0.18 %	6.86 % ± 0.57 %	5.99 % ± 0.28 %	0.00 % ± 0.00 %
M+3	0.42 % ± 0.03 %		2.93 % ± 0.22 %		3.27 % ± 0.06 %	0.60 % ± 0.09 %	9.26 % ± 0.35 %	0.00 % ± 0.00 %
M+4					0.12 % ± 0.03 %	0.12 % ± 0.07 %	0.47 % ± 0.10 %	24.61 % ± 8.75 %
M+5					0.12 % ± 0.03 %	0.03 % ± 0.03 %	0.28 % ± 0.07 %	

6 mM [U-$^{13}C_5$]glutamate *GP1562*								
	Fumaric acid	Glycerol	Methionine	Serine	Threonine	Phenylalanine	Malic acid	Aspartate
M+1	3.26 % ± 0.14 %	0.05 % ± 0.09 %	5.04 % ± 0.62 %	1.19 % ± 0.41 %	4.50 % ± 0.65 %	0.02 % ± 0.05 %	3.64 % ± 1.67 %	5.47 % ± 0.56 %
M+2	5.00 % ± 0.19 %	0.00 % ± 0.00 %	3.65 % ± 0.47 %	0.75 % ± 0.21 %	5.31 % ± 0.52 %	0.20 % ± 0.19 %	3.63 % ± 0.78 %	4.74 % ± 0.35 %
M+3	0.90 % ± 0.06 %	0.22 % ± 0.18 %	0.65 % ± 0.22 %	0.25 % ± 0.14 %	0.71 % ± 0.25 %	0.29 % ± 0.21 %	1.48 % ± 1.13 %	1.05 % ± 0.16 %

M+4	9.33 % ± 0.21 %		7.28 % ± 0.61 %		9.30 % ± 0.50 %	0.12 % ± 0.09 %	7.10 % ± 0.47 %	8.81 % ± 0.27 %
M+5			1.49 % ± 0.33 %			0.41 % ± 0.22 %		
M+6						0.10 % ± 0.14 %		
M+7						0.00 % ± 0.00 %		
M+8						0.07 % ± 0.12 %		
M+9						0.00 % ± 0.00 %		

6 mM [U-$^{13}C_5$]glutamate *GP1562*					
	Glutamate	Palmitate	Oelic acid	Stearic acid	Citric acid
M+1	4.16 % ± 0.28 %	0.00 % ± 0.00 %	1.32 % ± 0.34 %	0.52 % ± 0.36 %	4.55 % ± 3.04 %
M+2	2.88 % ± 0.17 %	0.66 % ± 0.24 %	9.87 % ± 0.68 %	0.77 % ± 0.34 %	6.14 % ± 3.01 %
M+3	3.62 % ± 0.05 %	0.28 % ± 0.04 %	1.12 % ± 0.28 %	0.17 % ± 0.04 %	2.97 % ± 3.27 %
M+4	0.44 % ± 0.03 %	0.22 % ± 0.07 %	3.77 % ± 0.21 %	0.43 % ± 0.17 %	7.14 % ± 1.99 %
M+5	9.51 % ± 0.28 %	0.03 % ± 0.01 %	0.73 % ± 0.21 %	0.06 % ± 0.02 %	0.25 % ± 0.54 %
M+6		0.03 % ± 0.02 %	1.65 % ± 0.19 %	0.19 % ± 0.08 %	0.48 % ± 0.97 %
M+7		0.00 % ± 0.00 %	0.31 % ± 0.11 %	0.02 % ± 0.01 %	

M+8		0.00 % ± 0.00 %	0.63 % ± 0.15 %	0.08 % ± 0.04 %	
M+9		0.00 % ± 0.00 %	0.10 % ± 0.06 %	0.01 % ± 0.01 %	
M+10		0.00 % ± 0.00 %	0.21 % ± 0.10 %	0.03 % ± 0.01 %	
M+11		0.00 % ± 0.00 %	0.04 % ± 0.04 %	0.01 % ± 0.01 %	
M+12		0.00 % ± 0.00 %	0.09 % ± 0.07 %	0.02 % ± 0.01 %	
M+13		0.00 % ± 0.00 %	0.03 % ± 0.02 %	0.01 % ± 0.00 %	
M+14		0.00 % ± 0.00 %	0.02 % ± 0.02 %	0.02 % ± 0.00 %	
M+15		0.01 % ± 0.00 %	0.01 % ± 0.02 %	0.01 % ± 0.00 %	
M+16		0.00 % ± 0.00 %	0.08 % ± 0.11 %	0.01 % ± 0.00 %	
M+17			0.03 % ± 0.04 %	0.00 % ± 0.00 %	
M+18			0.06 % ± 0.06 %	0.00 % ± 0.00 %	

Supplemental Table S69 Relative fractions of isotopologues (mol%) of sugars from experiments with *NCIB3610*, *GP921* and *GP1562* grown on Msgg agar plate supplemented with 6 mM [U-$^{13}C_5$]glutamate. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three independent experiments are shown.

6 mM [U-$^{13}C_5$]glutamate *NCIB3610*			
	Glucose in glycogen	Glucosamine	Muramic acid
M+1	1.34 % ± 0.27 %	1.49 % ± 0.76 %	6.32 % ± 1.18 %
M+2	0.45 % ± 0.09 %	2.02 % ± 1.75 %	2.45 % ± 0.65 %

M+3	0.56 % ± 0.07 %	0.04 % ± 0.06 %	0.13 % ± 0.25 %
M+4	0.03 % ± 0.02 %	0.05 % ± 0.08 %	0.36 % ± 0.42 %
M+5	0.02 % ± 0.00 %	0.03 % ± 0.03 %	0.00 % ± 0.00 %
M+6	0.03 % ± 0.02 %	0.01 % ± 0.01 %	0.63 % ± 0.71 %

6 mM [U-$^{13}C_5$]glutamate *GP921*			
	Glucose in glycogen	Glucosamine	Muramic acid
M+1	0.13 % ± 0.30 %	0.50 % ± 0.47 %	2.69 % ± 1.28 %
M+2	0.09 % ± 0.11 %	0.38 % ± 0.25 %	1.52 % ± 0.56 %
M+3	0.20 % ± 0.34 %	0.00 % ± 0.00 %	0.53 % ± 0.46 %
M+4	0.03 % ± 0.04 %	0.00 % ± 0.01 %	0.25 % ± 0.15 %
M+5	0.03 % ± 0.04 %	0.01 % ± 0.01 %	0.12 % ± 0.11 %
M+6	0.32 % 0.38 %	0.00 % 0.00 %	0.00 % 0.00 %

6 mM [U-$^{13}C_5$]glutamate *GP1562*			
	Glucose in glycogen	Glucosamine	Muramic acid
M+1	0.18 % ± 0.24 %	0.51 % ± 0.45 %	3.63 % ± 1.73 %
M+2	0.08 % ± 0.10 %	0.68 % ± 0.46 %	2.07 % ± 0.33 %

M+3	0.22 % ± 0.11 %	0.03 % ± 0.05 %	0.86 % ± 0.60 %
M+4	0.02 % ± 0.01 %	0.04 % ± 0.04 %	0.37 % ± 0.22 %
M+5	0.05 % ± 0.02 %	0.06 % ± 0.05 %	0.08 % ± 0.09 %
M+6	0.21 % ± 0.21 %	0.02 % ± 0.03 %	0.23 % ± 0.20 %

Supplemental Table S70 p value of metabolites from [U-$^{13}C_6$]glucose agar plate

P value of metabolites from experiments with *NCIB3610*, *GP921* and *GP1562* grown on Msgg agar plate supplemented with 9 mM [U-$^{13}C_6$]glucose. Mean and SD from six independent experiments are shown.

	GP921/NCIB3610	GP1562/NCIB3610	GP1562/GP921
Ala	4.11E-17	6.73E-26	2.80E-18
Asp	1.37E-20	1.86E-23	1.01E-03
DAP	1.56E-12	4.57E-24	2.57E-10
Glu	3.95E-34	1.33E-08	5.19E-37
Gly	9.35E-04	6.00E-23	1.72E-30
His	2.87E-22	3.93E-28	7.21E-33
Ile	1.46E-19	1.68E-25	3.52E-15
Leu	3.07E-18	3.15E-16	7.59E-11
Lys	4.00E-16	3.29E-20	5.24E-05
Met	1.89E-01	2.86E-04	4.97E-02
Phe	4.59E-13	6.07E-14	5.66E-03
Pro	2.03E-23	5.09E-08	3.10E-26
Ser	8.68E-01	5.99E-22	5.09E-26
Thr	6.02E-08	1.23E-21	8.44E-02
Tyr	1.91E-08	4.32E-28	8.59E-25
Val	2.41E-16	2.95E-26	2.03E-18
Lactate	1.59E-01	2.37E-07	2.99E-02
Glycolic acid	7.87E-07	8.79E-01	7.32E-05
Alanine	4.17E-07	1.92E-07	7.45E-01
Glycine	1.81E-10	2.13E-15	1.94E-20
Valine	5.92E-01	5.31E-02	2.40E-01
leucine	1.86E-22	1.83E-08	7.04E-03
Isoleucine	1.13E-06	6.56E-09	9.68E-07
Succinic acid	2.43E-03	1.30E-08	1.21E-04
Fumaric acid	1.42E-06	8.97E-03	2.01E-05
Glycerol	3.05E-01	2.22E-06	2.20E-04
Methionine	9.22E-01	5.55E-02	6.61E-02
Serine	5.12E-01	1.12E-04	7.98E-05
Threonine	6.84E-02	6.35E-06	4.30E-05
Phenylalanine	1.61E-10	4.98E-02	4.61E-04
Malic acid	1.78E-04	1.07E-01	1.32E-01
Aspartate	3.30E-01	2.28E-02	2.82E-02
Glutamate	4.52E-01	8.04E-02	6.87E-02

Glycine	6.88E-21	1.75E-03	5.48E-21
Glucose in glycogen	8.35E-01	3.74E-09	4.25E-09
Glucosamine	7.02E-01	1.27E-19	3.29E-18
Muramic acid	1.03E-04	1.12E-19	5.29E-14

Supplemental Table S71 p value of metabolites from [U-$^{13}C_5$]glutamate agar plate

P value of metabolites from experiments with *NCIB3610*, *GP921* and *GP1562* grown on Msgg agar plate supplemented with 6 mM [U-$^{13}C_5$]glutamate. Mean and SD from three independent experiments are shown.

	NCIB3610/GP921	NCIB3610/GP1562	GP921/GP1562
Ala	1.46E-15	1.42E-13	2.50E-07
Asp	5.43E-09	1.13E-11	6.14E-09
DAP	3.72E-07	2.28E-08	2.14E-07
Glu	2.86E-11	2.16E-10	3.46E-02
Gly	1.15E-09	9.05E-01	1.43E-10
His	8.61E-02	4.08E-01	2.88E-07
Ile	9.95E-04	7.58E-10	6.73E-08
Leu	3.89E-08	5.19E-09	1.33E-02
Lys	1.06E-07	6.00E-07	1.24E-12
Met	1.84E-06	6.67E-05	1.08E-09
Phe	7.58E-17	5.52E-12	1.03E-08
Pro	2.69E-05	3.21E-03	2.02E-06
Ser	3.38E-11	3.16E-01	8.75E-09
Thr	4.65E-05	5.20E-15	1.06E-13
Tyr	2.21E-09	8.43E-10	5.89E-20
Val	6.45E-12	3.26E-09	1.05E-07
Lactate	4.87E-01	3.93E-07	1.50E-11
Glycolic acid	1.10E-01	1.88E-01	8.63E-01
Alanine	8.52E-01	1.14E-09	2.47E-10
Glycine	1.16E-10	1.90E-11	5.79E-01
Valine	5.68E-14	6.15E-01	3.54E-16
leucine	2.48E-13	1.53E-01	1.97E-07
Isoleucine	1.09E-04	3.97E-04	6.26E-02
Succinic acid	5.84E-05	1.09E-06	4.48E-06
Fumaric acid	9.69E-10	9.27E-08	1.77E-01
Glycerol	2.14E-04	1.38E-03	5.39E-01
Methionine	8.59E-01	8.92E-08	6.32E-08

Serine	2.00E-14	1.67E-15	2.56E-07
Threonine	2.62E-02	2.85E-04	1.59E-02
Phenylalanine	3.32E-11	3.94E-05	2.27E-02
Malic acid	2.22E-05	2.02E-02	8.66E-06
Aspartate	2.65E-14	1.92E-10	4.00E-07
Glutamate	1.42E-13	6.46E-11	1.82E-07
Palmitate	1.03E-05	2.09E-06	8.36E-09
Oelic acid	2.77E-02	3.17E-05	8.54E-04
Stearic acid	2.05E-04	1.00E-04	4.37E-04
Glucose in glycogen	3.78E-01	1.15E-02	6.99E-01
Glucosamine	3.53E-03	1.82E-02	6.33E-02
Muramic acid	4.24E-04	8.04E-02	1.06E-03

Supplemental Table S72 Composition of CSE medium in the [U-$^{13}C_6$]glucose labelled experiments.

Component	**Final concentration**
KH_2PO_4	30 mM
K_2HPO_4 x 3 H_2O	70 mM
$(NH_4)_2SO_4$	25 mM
$MnSO_4$ x 3 H_2O	10 µM
$MgSO_4$ x 7 H_2O	0.5 mM
Potassium L-glutamate	8 mg/ml
L-Tryptophan	50 mg/l
Ammonium iron citrate	22 mg/l
Sodium succinate	6 mg/ml
D-glucose	13.9 mM
D-[U-$^{13}C_6$]glucose	13.4 mM

Supplemental Table S73 Composition of pre-culture LB-medium

LB medium (1 l)	Tryptone	10 g
	Yeast extract	5 g
	NaCl	10 g

Supplemental Table S74 Composition of MSgg agar plate in the [U-$^{13}C_6$]glucose and [U-$^{13}C_5$]glutamate labelled experiments.

MSgg medium

Component	Final concentration
Potassium phosphate buffer pH 7.0	5 mM
MOPS pH 7.0	100 mM
Glycerol	68 mM
Thiamine	2 µM
Potassium glutamate	24.6 mM
L-Tryptophan	100 mg/l
L-Phenylalanine	100 mg/l
$MgCl_2$	2 mM
$CaCl_2$	700 µM
$MnCl_2$	50 µM
$FeCl_3$ x 6 H_2O^4	50 µM
$ZnCl_2$	1 µM

MSgg glucose / ^{13}C-glucose medium

Component	Final concentration
Potassium phosphate buffer pH 7.0	5 mM
MOPS pH 7.0	100 mM
Glycerol	68 mM
Glucose	18.5 mM
D-[U-$^{13}C_6$]glucose	9.3 mM
Thiamine	2 µM
Potassium glutamate	24.6 mM
L-Tryptophan	100 mg/l
L-Phenylalanine	100 mg/l

$MgCl_2$	2 mM
$CaCl_2$	700 µM
$MnCl_2$	50 µM
$FeCl_3$ x 6 H_2O[4]	50 µM
$ZnCl_2$	1 µM

MSgg glutamate / ^{13}C-glutamate medium

Component	Final concentration
Potassium phosphate buffer pH 7.0	5 mM
MOPS pH 7.0	100 mM
Glycerol	68 mM
Thiamine	2 µM
Potassium glutamate	19.7 mM
L-[U-$^{13}C_5$]glutamate	5.4 mM
L-Tryptophan	100 mg/l
L-Phenylalanine	100 mg/l
$MgCl_2$	2 mM
$CaCl_2$	700 µM
$MnCl_2$	50 µM
$FeCl_3$ x 6 H_2O	50 µM
$ZnCl_2$	1 µM

Supplemental Tables S75 ^{13}C-Excess (mol%) of protein-bound amino acids from [U-$^{13}C_6$]glucose labelled *immortal brown adipose tissue (IBAT)*

^{13}C-Excess (mol%) of protein-bound amino acids from experiments with *immortal brown adipose tissue* grown in DMEM medium supplemented with 5 mM [U-$^{13}C_6$]glucose. Mean and SD from three technical replicates are shown.

	con. 1	**iso. 1**	**con. 2**	**iso. 2**
Ala-260	0.39% ± 0.05%	0.41% ± 0.05%	0.66% ± 0.07%	0.59% ± 0.05%
Asp-418	0.33% ± 0.04%	0.41% ± 0.09%	0.47% ± 0.11%	0.47% ± 0.06%
Glu-432	0.64% ± 0.02%	0.64% ± 0.03%	0.67% ± 0.09%	0.62% ± 0.02%
Gly-246	0.45% ± 0.02%	0.53% ± 0.09%	0.64% ± 0.04%	0.58% ± 0.03%
His-440	0.16% ± 0.00%	0.13% ± 0.04%	0.52% ± 0.05%	0.25% ± 0.07%
Ile-274	0.17% ± 0.03%	0.22% ± 0.03%	0.18% ± 0.02%	0.19% ± 0.02%
Leu-274	0.21% ± 0.05%	0.26% ± 0.03%	0.24% ± 0.00%	0.23% ± 0.03%
Lys-431	0.05% ± 0.06%	0.11% ± 0.01%	0.17% ± 0.04%	0.15% ± 0.06%
Met-320	0.35% ± 0.26%	0.37% ± 0.19%	0.09% ± 0.02%	0.25% ± 0.02%
Phe-336	0.15% ± 0.04%	0.19% ± 0.03%	0.19% ± 0.04%	0.24% ± 0.04%
Pro-286	0.27% ± 0.02%	0.37% ± 0.01%	0.38% ± 0.03%	0.34% ± 0.03%
Ser-390	0.21% ± 0.03%	0.28% ± 0.04%	0.33% ± 0.01%	0.29% ± 0.08%
Thr-404	0.79% ± 0.05%	0.75% ± 0.04%	0.83% ± 0.06%	0.79% ± 0.07%
Tyr-466	0.10% ± 0.04%	0.15% ± 0.04%	0.12% ± 0.05%	0.14% ± 0.04%
Val-288	0.14% ± 0.02%	0.20% ± 0.06%	0.26% ± 0.03%	0.23% ± 0.03%

Supplemental Table S76 Relative fractions of isotopologues (mol%) of protein-bound amino acids from experiments with *immortal brown adipose tissue* grown in DMEM medium supplemented with 5 mM [U-$^{13}C_6$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three technical replicates are shown.

5 mM [U-$^{13}C_6$]glucose *con. 1*								
	Ala-260	**Asp-418**	**Glu-432**	**Gly-246**	**His-440**	**Ile-274**	**Leu-274**	**Lys-431**
M+1	0.46 % ± 0.23 %	1.30 % ± 0.17 %	1.35 % ± 0.21 %	0.57 % ± 0.04 %	0.00 % ± 0.00 %	0.13 % ± 0.12 %	0.67 % ± 0.18 %	0.02 % ± 0.02 %
M+2	0.14 % ± 0.05 %	0.00 % ± 0.00 %	0.91 % ± 0.13 %	0.16 % ± 0.02 %	0.00 % ± 0.00 %	0.35 % ± 0.03 %	0.16 % ± 0.04 %	0.00 % ± 0.00 %
M+3	0.15 % ± 0.01 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %		0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.01 % ± 0.01 %	0.10 % ± 0.11 %
M+4		0.00 % ± 0.00 %	0.00 % ± 0.00 %		0.15 % ± 0.03 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+5			0.00 % ± 0.00 %		0.07 % ± 0.03 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+6					0.00 % ± 0.00 %			0.00 % ± 0.00 %

5 mM [U-$^{13}C_6$]glucose *con. 1*							
	Met-320	**Phe-336**	**Pro-286**	**Ser-390**	**Thr-404**	**Tyr-466**	**Val-288**
M+1	0.00% ± 0.00%	0.80% ± 0.43%	0.17% ± 0.05%	0.13% ± 0.23%	2.60% ± 0.38%	0.53% ± 0.46%	0.55% ± 0.08%
M+2	0.01% ± 0.01%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%	0.27% ± 0.12%	0.00% ± 0.00%	0.08% ± 0.07%
M+3	0.12% ± 0.22%	0.00% ± 0.00%	0.15% ± 0.01%	0.17% ± 0.07%	0.00% ± 0.00%	0.01% ± 0.02%	0.00% ± 0.00%

M+4	0.00% ± 0.00%	0.13% ± 0.02%	0.18% ± 0.02%		0.00% ± 0.00%	0.00% ± 0.00%	0.01% ± 0.01%
M+5	0.27% ± 0.15%	0.01% ± 0.01%	0.00% ± 0.00%			0.07% ± 0.00%	0.00% ± 0.00%
M+6		0.00% ± 0.00%				0.00% ± 0.00%	
M+7		0.00% ± 0.00%				0.00% ± 0.00%	
M+8		0.00% ± 0.00%				0.00% ± 0.00%	
M+9		0.00% ± 0.00%				0.00% ± 0.00%	

5 mM [U-$^{13}C_6$]glucose *iso. 1*								
	Ala-260	**Asp-418**	**Glu-432**	**Gly-246**	**His-440**	**Ile-274**	**Leu-274**	**Lys-431**
M+1	0.70 % ± 0.16 %	1.62 % ± 0.35 %	1.63 % ± 0.32 %	0.75 % ± 0.09 %	0.44 % ± 0.23 %	0.21 % ± 0.20 %	0.86 % ± 0.13 %	0.06 % ± 0.05 %
M+2	0.21 % ± 0.01 %	0.00 % ± 0.00 %	0.78 % ± 0.08 %	0.15 % ± 0.04 %	0.00 % ± 0.00 %	0.44 % ± 0.03 %	0.20 % ± 0.05 %	0.00 % ± 0.00 %
M+3	0.04 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %		0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.01 % ± 0.01 %	0.20 % ± 0.00 %
M+4		0.00 % ± 0.00 %	0.00 % ± 0.00 %		0.00 % ± 0.01 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+5			0.00 % ± 0.00 %		0.06 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+6					0.00 % ± 0.00 %			0.00 % ± 0.00 %

5 mM [U-$^{13}C_6$]glucose *iso. 1*							
	Met-320	**Phe-336**	**Pro-286**	**Ser-390**	**Thr-404**	**Tyr-466**	**Val-288**
M+1	0.03% ± 0.03%	1.24% ± 0.25%	0.50% ± 0.05%	0.47% ± 0.08%	2.46% ± 0.32%	1.12% ± 0.39%	0.86% ± 0.11%
M+2	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%	0.27% ± 0.07%	0.00% ± 0.01%	0.07% ± 0.11%
M+3	0.06% ± 0.10%	0.00% ± 0.00%	0.14% ± 0.01%	0.13% ± 0.01%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+4	0.10% ± 0.18%	0.12% ± 0.01%	0.24% ± 0.01%		0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+5	0.24% ± 0.21%	0.00% ± 0.00%	0.00% ± 0.00%			0.05% ± 0.00%	0.00% ± 0.00%
M+6		0.00% ± 0.00%				0.00% ± 0.00%	
M+7		0.00% ± 0.00%				0.00% ± 0.00%	
M+8		0.00% ± 0.00%				0.00% ± 0.00%	
M+9		0.00% ± 0.00%				0.00% ± 0.00%	

5 mM [U-$^{13}C_6$]glucose *con. 2*								
	Ala-260	**Asp-418**	**Glu-432**	**Gly-246**	**His-440**	**Ile-274**	**Leu-274**	**Lys-431**
M+	1.02 ± 0.26	1.88 ± 0.44	1.62 ± 0.45	0.80 ± 0.08	0.66 ± 0.32	0.06 ± 0.11	0.61 ± 0.10	0.40 ± 0.30

1	% %	% %	% %	% %	% %	% %	% %	% %
M+2	0.29 % ± 0.07 %	0.00 % ± 0.00 %	0.86 % ± 0.07 %	0.24 % ± 0.02 %	0.00 % ± 0.00 %	0.42 % ± 0.03 %	0.26 % ± 0.05 %	0.00 % ± 0.00 %
M+3	0.13 % ± 0.01 %	0.00 % ± 0.00 %	0.00 % ± 0.01 %		0.04 % ± 0.07 %	0.00 % ± 0.00 %	0.02 % ± 0.01 %	0.20 % ± 0.02 %
M+4		0.00 % ± 0.00 %	0.00 % ± 0.00 %		0.49 % ± 0.05 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+5			0.00 % ± 0.00 %		0.08 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+6					0.00 % ± 0.00 %			0.00 % ± 0.00 %

5 mM [U-$^{13}C_6$]glucose *con. 2*							
	Met-320	**Phe-336**	**Pro-286**	**Ser-390**	**Thr-404**	**Tyr-466**	**Val-288**
M+1	0.00% ± 0.00%	1.22% ± 0.36%	0.61% ± 0.15%	0.34% ± 0.28%	2.72% ± 0.42%	0.77% ± 0.55%	1.03% ± 0.13%
M+2	0.07% ± 0.12%	0.00% ± 0.00%	0.00% ± 0.00%	0.03% ± 0.06%	0.29% ± 0.09%	0.04% ± 0.06%	0.14% ± 0.01%
M+3	0.00% ± 0.00%	0.00% ± 0.00%	0.14% ± 0.04%	0.19% ± 0.06%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+4	0.00% ± 0.00%	0.12% ± 0.01%	0.22% ± 0.03%		0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+5	0.06% ± 0.03%	0.00% ± 0.00%	0.00% ± 0.00%			0.05% ± 0.01%	0.00% ± 0.00%
M+6		0.00% ± 0.00%				0.00% ± 0.00%	
M+7		0.00% ± 0.00%				0.00% ± 0.00%	

M+8		0.00% ± 0.00%				0.00% ± 0.00%	
M+9		0.00% ± 0.00%				0.00% ± 0.00%	

5 mM [U-$^{13}C_6$]glucose *iso. 2*								
	Ala-260	**Asp-418**	**Glu-432**	**Gly-246**	**His-440**	**Ile-274**	**Leu-274**	**Lys-431**
M+1	1.04 % ± 0.15 %	1.86 % ± 0.26 %	1.37 % ± 0.14 %	0.77 % ± 0.02 %	0.84 % ± 0.50 %	0.15 % ± 0.16 %	0.67 % ± 0.04 %	0.22 % ± 0.34 %
M+2	0.20 % ± 0.01 %	0.00 % ± 0.00 %	0.87 % ± 0.01 %	0.20 % ± 0.02 %	0.00 % ± 0.00 %	0.38 % ± 0.03 %	0.22 % ± 0.05 %	0.00 % ± 0.00 %
M+3	0.11 % ± 0.00 %	0.01 % ± 0.01 %	0.00 % ± 0.00 %		0.02 % ± 0.03 %	0.01 % ± 0.01 %	0.01 % ± 0.01 %	0.23 % ± 0.04 %
M+4		0.00 % ± 0.00 %	0.00 % ± 0.00 %		0.09 % ± 0.01 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+5			0.00 % ± 0.00 %		0.06 % ± 0.01 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+6					0.00 % ± 0.00 %			0.00 % ± 0.00 %

5 mM [U-$^{13}C_6$]glucose *iso. 2*							
	Met-320	**Phe-336**	**Pro-286**	**Ser-390**	**Thr-404**	**Tyr-466**	**Val-288**
M+1	0.01% ± 0.01%	1.63% ± 0.30%	0.41% ± 0.18%	0.37% ± 0.34%	2.72% ± 0.32%	1.10% ± 0.36%	0.89% ± 0.07%

M+2	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%	0.01% ± 0.01%	0.22% ± 0.13%	0.03% ± 0.05%	0.13% ± 0.07%
M+3	0.00% ± 0.00%	0.00% ± 0.00%	0.12% ± 0.02%	0.17% ± 0.03%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+4	0.06% ± 0.06%	0.12% ± 0.01%	0.24% ± 0.01%		0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+5	0.20% ± 0.05%	0.00% ± 0.00%	0.00% ± 0.00%			0.03% ± 0.03%	0.00% ± 0.00%
M+6		0.00% ± 0.00%				0.00% ± 0.00%	
M+7		0.00% ± 0.00%				0.00% ± 0.00%	
M+8		0.00% ± 0.00%				0.00% ± 0.00%	
M+9		0.00% ± 0.00%				0.00% ± 0.00%	

Supplemental Tables S77 ^{13}C-Excess (mol%) of polar metabolites from [U-$^{13}C_6$]glucose labelled *immortal brown adipose tissue (IBAT)*

^{13}C-Excess (mol%) of polar metabolites from experiments with *immortal brown adipose tissue* grown in DMEM medium supplemented with 5 mM [U-$^{13}C_6$]glucose. Mean and SD from three technical replicates are shown.

	con. 1	**iso. 1**	**con. 2**	**iso. 2**
Lactate-261	9.67 % ± 0.07 %	9.63 % ± 0.02 %	10.91 % ± 0.03 %	10.61 % ± 0.05 %
Glycolic acid-247	1.98 % ± 0.19 %	0.53 % ± 0.18 %	1.50 % ± 0.96 %	2.12 % ± 0.38 %
Ala-260	4.90 % ± 0.01 %	4.41 % ± 0.06 %	2.70 % ± 0.09 %	2.50 % ± 0.06 %
Gly-246	0.22 % ± 0.06 %	0.16 % ± 0.05 %	0.27 % ± 0.17 %	0.18 % ± 0.11 %
Oxalic acid-261	1.28 % ± 2.21 %	0.02 % ± 0.04 %	0.90 % ± 0.97 %	1.93 % ± 1.57 %
3-Hydroxybutyrate-275	7.47 % ± 0.72 %	4.00 % ± 0.96 %	5.85 % ± 5.25 %	2.52 % ± 0.58 %
Val-288	0.03 % ± 0.03 %	0.06 % ± 0.01 %	0.12 % ± 0.04 %	0.06 % ± 0.04 %
Leu-274	0.25 % ± 0.07 %	0.14 % ± 0.00 %	0.13 % ± 0.02 %	0.16 % ± 0.05 %

Ile-274	0.47 % ± 0.05 %	0.43 % ± 0.03 %	0.40 % ± 0.04 %	0.36 % ± 0.11 %
Succinate-289	2.78 % ± 1.04 %	3.28 % ± 2.94 %	0.93 % ± 0.78 %	0.15 % ± 0.15 %
Fumarate-287	8.46 % ± 0.13 %	4.59 % ± 0.12 %	9.63 % ± 1.58 %	7.42 % ± 0.90 %
Glycerol-377	0.18 % ± 0.13 %	0.49 % ± 0.07 %	0.38 % ± 0.21 %	0.70 % ± 0.21 %
Met-320	0.80 % ± 0.09 %	1.20 % ± 0.31 %	1.50 % ± 0.52 %	1.55 % ± 0.25 %
Ser-390	0.58 % ± 0.09 %	0.52 % ± 0.07 %	0.47 % ± 0.14 %	0.66 % ± 0.04 %
Thr-404	0.09 % ± 0.09 %	0.08 % ± 0.02 %	0.11 % ± 0.14 %	0.09 % ± 0.06 %
Phe-336	2.81 % ± 0.13 %	4.89 % ± 0.46 %	2.11 % ± 3.62 %	4.81 % ± 4.03 %
Malate-419	4.16 % ± 0.41 %	3.48 % ± 0.93 %	4.59 % ± 3.52 %	4.18% ± 1.13%
Asp-418	3.00 % ± 0.23 %	3.31 % ± 0.41 %	2.67 % ± 1.13 %	3.79% ± 0.13%
Glu-432	2.93 % ± 0.09 %	2.72 % ± 0.10 %	3.33 % ± 0.06 %	2.86% ± 0.07%
Palmitic acid-313	0.04 % ± 0.00 %	0.03 % ± 0.01 %	0.02 % ± 0.01 %	0.03% ± 0.02%
Oleic acid-339	0.67 % ± 0.02 %	0.69 % ± 0.01 %	0.66 % ± 0.02 %	0.67% ± 0.02%
Stearic acid-341	0.28 % ± 0.02 %	0.32 % ± 0.01 %	0.28 % ± 0.02 %	0.29% ± 0.01%

Supplemental Table S78 Relative fractions of isotopologues (mol%) of polar metabolites from experiments with *immortal brown adipose tissue* grown in DMEM medium supplemented with 5 mM [U-$^{13}C_6$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three technical replicates are shown.

5 mM [U-$^{13}C_6$]glucose *con. 1*					
	Ala-260	**Asp-418**	**Glu-432**	**Phe-336**	**Ser-390**
M+1	0.36 % ± 0.06 %	1.95 % ± 0.33 %	1.37 % ± 0.16 %	0.30 % ± 0.33 %	0.00 % ± 0.00 %
M+2	0.43 % ± 0.03 %	2.26 % ± 0.19 %	5.06 % ± 0.12 %	0.00 % ± 0.00 %	0.42 % ± 0.27 %
M+3	4.49 % ± 0.03 %	1.72 % ± 0.06 %	0.70 % ± 0.02 %	4.97 % ± 0.04 %	0.30 % ± 0.09 %
M+4		0.09 % ± 0.10 %	0.08 % ± 0.01 %	0.09 % ± 0.09 %	
M+5			0.15 % ± 0.02 %	1.68 % ± 0.34 %	
M+6				0.11 % ± 0.07 %	
M+7				0.09 % ± 0.09 %	
M+8				0.00 % ± 0.00 %	
M+9				0.00 % ± 0.01 %	

5 mM [U-$^{13}C_6$]glucose *con. 1*						
	3-Hydroxybutyrate-275	**Fumarate-287**	**Glycolic acid-247**	**Lactate-261**	**Malate-419**	**Succinate-289**
M+1	1.19% ± 2.06 %	0.00 % ± 0.00 %	0.20 % ± 0.30 %	0.09 % ± 0.08 %	1.69 % ± 1.69%	0.00 % ± 0.00 %
M+2	14.20 % ± 1.21 %	1.87 % ± 0.24 %	1.88 % ± 0.11 %	0.81 % ± 0.03 %	2.04 % ± 0.69%	0.00 % ± 0.00 %
M+3	0.09% ± 0.16 %	2.47 % ± 0.19 %		9.10 % ± 0.03 %	3.45 % ± 0.90%	1.15 % ± 0.23 %
M+4	0.00% ± 0.00 %	5.67 % ± 0.29 %			0.13 % ± 0.22%	1.92 % ± 1.01 %

5 mM [U-$^{13}C_6$]glucose *con. 1*		
	Oleic acid-339	**Stearic acid-341**
M+1	1.11% ± 0.20%	2.98% ± 0.29%
M+2	3.87% ± 0.05%	0.14% ± 0.06%
M+3	0.00% ± 0.00%	0.00% ± 0.00%
M+4	0.03% ± 0.01%	0.00% ± 0.00%
M+5	0.01% ± 0.00%	0.01% ± 0.00%
M+6	0.01% ± 0.01%	0.01% ± 0.00%
M+7	0.01% ± 0.01%	0.00% ± 0.00%

M+8	0.00% ± 0.00%	0.00% ± 0.00%
M+9	0.00% ± 0.00%	0.00% ± 0.00%
M+10	0.01% ± 0.00%	0.01% ± 0.00%
M+11	0.00% ± 0.00%	0.00% ± 0.00%
M+12	0.01% ± 0.00%	0.03% ± 0.00%
M+13	0.01% ± 0.00%	0.02% ± 0.00%
M+14	0.05% ± 0.01%	0.04% ± 0.00%
M+15	0.03% ± 0.00%	0.02% ± 0.00%
M+16	0.04% ± 0.00%	0.01% ± 0.00%
M+17	0.02% ± 0.00%	0.00% ± 0.00%
M+18	0.02% ± 0.01%	0.00% ± 0.00%

5 mM [U-$^{13}C_6$]glucose *iso. 1*					
	Ala-260	**Asp-418**	**Glu-432**	**Phe-336**	**Ser-390**
M+1	0.37% ± 0.11%	3.74% ± 0.69%	3.00% ± 0.39%	0.68% ± 0.27%	0.00% ± 0.00%
M+2	0.36% ± 0.06%	2.77% ± 0.27%	3.84% ± 0.09%	0.00% ± 0.00%	0.45% ± 0.14%
M+3	4.05% ± 0.01%	1.01% ± 0.25%	0.64% ± 0.05%	8.15% ± 0.69%	0.22% ± 0.04%
M+4		0.23% ± 0.36%	0.06% ± 0.02%	0.00% ± 0.01%	

M+5			0.15% ± 0.02%	2.97% ± 0.40%	
M+6				0.35% ± 0.12%	
M+7				0.14% ± 0.16%	
M+8				0.08% ± 0.09%	
M+9				0.04% ± 0.05%	

5 mM [U-$^{13}C_6$]glucose *iso. 1*						
	3-Hydroxybutyrate-275	**Fumarate-287**	**Glycolic acid-247**	**Lactate-261**	**Malate-419**	**Succinate-289**
M+1	0.77% ± 1.33%	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.14 % ± 0.07 %	3.15 % ± 0.71%	0.00 % ± 0.00 %
M+2	5.93% ± 0.91%	2.01 % ± 0.16 %	0.53 % ± 0.18 %	0.79 % ± 0.08 %	2.58 % ± 1.15%	0.31 % ± 0.32 %
M+3	0.05% ± 0.08%	1.13 % ± 0.20 %		9.06 % ± 0.03 %	1.62 % ± 0.57%	0.71 % ± 0.63 %
M+4	0.80% ± 1.39%	2.74 % ± 0.14 %			0.19 % ± 0.33%	2.59 % ± 2.58 %

5 mM [U-$^{13}C_6$]glucose *iso. 1*		
	Oleic acid-339	**Stearic acid-341**
M+1	1.68 % ± 0.21 %	2.63 % ± 0.26 %

M+2	3.83 %	±	0.03 %	0.13 %	±	0.03 %
M+3	0.00 %	±	0.00 %	0.00 %	±	0.00 %
M+4	0.03 %	±	0.00 %	0.00 %	±	0.00 %
M+5	0.01 %	±	0.01 %	0.01 %	±	0.00 %
M+6	0.01 %	±	0.00 %	0.01 %	±	0.01 %
M+7	0.01 %	±	0.00 %	0.00 %	±	0.00 %
M+8	0.00 %	±	0.00 %	0.01 %	±	0.01 %
M+9	0.00 %	±	0.00 %	0.00 %	±	0.00 %
M+10	0.00 %	±	0.00 %	0.01 %	±	0.00 %
M+11	0.00 %	±	0.00 %	0.00 %	±	0.00 %
M+12	0.02 %	±	0.00 %	0.08 %	±	0.02 %
M+13	0.01 %	±	0.00 %	0.02 %	±	0.00 %
M+1	0.05	±	0.00	0.04	±	0.00

4	% %	% %
M+15	0.03 % ± 0.00 %	0.02 % ± 0.00 %
M+16	0.04 % ± 0.00 %	0.01 % ± 0.00 %
M+17	0.02 % ± 0.00 %	0.00 % ± 0.00 %
M+18	0.01 % ± 0.00 %	0.01 % ± 0.00 %

5 mM [U-$^{13}C_6$]glucose *con. 2*					
	Ala-260	**Asp-418**	**Glu-432**	**Phe-336**	**Ser-390**
M+1	0.09% ± 0.09%	3.18% ± 0.89%	3.03% ± 0.50%	0.38% ± 0.29%	0.00% ± 0.00%
M+2	0.18% ± 0.03%	0.88% ± 0.84%	5.27% ± 0.11%	0.00% ± 0.00%	0.33% ± 0.29%
M+3	2.55% ± 0.05%	1.61% ± 1.28%	0.89% ± 0.14%	3.67% ± 6.35%	0.25% ± 0.07%
M+4		0.23% ± 0.22%	0.07% ± 0.05%	0.02% ± 0.04%	
M+5			0.03% ± 0.04%	1.18% ± 2.04%	
M+6				0.21% ± 0.37%	
M+7				0.03% ± 0.05%	
M+8				0.00% ± 0.00%	

M+9				0.02% ± 0.03%	

5 mM [U-$^{13}C_6$]glucose *con. 2*						
	3-Hydroxybutyrate-275	**Fumarate-287**	**Glycolic acid-247**	**Lactate-261**	**Malate-419**	**Succinate-289**
M+1	3.11% ± 2.87%	0.00% ± 0.00%	0.00% ± 0.00%	0.03% ± 0.03%	1.98% ± 3.42%	0.00% ± 0.00%
M+2	5.77% ± 4.80%	1.30% ± 0.55%	1.50% ± 0.96%	0.82% ± 0.02%	0.99% ± 1.71%	0.00% ± 0.00%
M+3	0.23% ± 0.39%	2.15% ± 0.61%		10.35% ± 0.01%	3.00% ± 2.85%	0.46% ± 0.38%
M+4	2.01% ± 2.98%	7.36% ± 1.45%			1.35% ± 1.29%	0.58% ± 0.51%

5 mM [U-$^{13}C_6$]glucose *con. 2*		
	Oleic acid-339	**Stearic acid-341**
M+1	1.59% ± 0.14%	2.70% ± 0.32%
M+2	3.80% ± 0.13%	0.14% ± 0.01%
M+3	0.00% ± 0.00%	0.00% ± 0.00%
M+4	0.03% ± 0.00%	0.00% ± 0.00%
M+5	0.01% ± 0.00%	0.01% ± 0.00%
M+6	0.00% ± 0.00%	0.01% ± 0.00%
M+7	0.00% ± 0.00%	0.00% ± 0.00%

M+8	0.00% ± 0.00%	0.01% ± 0.00%
M+9	0.00% ± 0.00%	0.00% ± 0.00%
M+10	0.00% ± 0.00%	0.01% ± 0.01%
M+11	0.00% ± 0.00%	0.00% ± 0.00%
M+12	0.01% ± 0.00%	0.06% ± 0.01%
M+13	0.01% ± 0.01%	0.02% ± 0.00%
M+14	0.05% ± 0.01%	0.04% ± 0.00%
M+15	0.03% ± 0.00%	0.01% ± 0.00%
M+16	0.04% ± 0.00%	0.01% ± 0.00%
M+17	0.01% ± 0.00%	0.00% ± 0.00%
M+18	0.01% ± 0.00%	0.00% ± 0.00%

5 mM [U-$^{13}C_6$]glucose *iso. 2*					
	Ala-260	**Asp-418**	**Glu-432**	**Phe-336**	**Ser-390**
M+1	0.14% ± 0.12%	2.53% ± 1.15%	3.13% ± 0.48%	0.59% ± 0.14%	0.00% ± 0.00%
M+2	0.22% ± 0.05%	2.76% ± 1.20%	4.34% ± 0.07%	0.01% ± 0.02%	0.50% ± 0.18%

M+3	2.30% ± 0.06%	1.99% ± 0.10%	0.65% ± 0.25%	8.30% ± 7.19%	0.32% ± 0.09%
M+4		0.28% ± 0.49%	0.10% ± 0.10%	0.09% ± 0.13%	
M+5			0.03% ± 0.05%	2.63% ± 2.29%	
M+6				0.59% ± 0.55%	
M+7				0.04% ± 0.07%	
M+8				0.03% ± 0.05%	
M+9				0.02% ± 0.02%	

5 mM [U-$^{13}C_6$]glucose *iso. 2*						
	3-Hydroxybutyrate-275	**Fumarate-287**	**Glycolic acid-247**	**Lactate-261**	**Malate-419**	**Succinate-289**
M+1	0.13% ± 0.22 %	0.00 % ± 0.00 %	0.26 % ± 0.36 %	0.09% ± 0.07%	3.39 % ± 2.39 %	0.00 % ± 0.00 %
M+2	4.98% ± 1.12 %	1.55 % ± 0.44 %	1.99 % ± 0.51 %	0.81% ± 0.06%	3.54 % ± 2.55 %	0.09 % ± 0.16 %
M+3	0.00% ± 0.00 %	2.11 % ± 0.64 %		10.04% ± 0.07%	0.91 % ± 0.90 %	0.13 % ± 0.23 %
M+4	0.00% ± 0.00 %	5.06 % ± 0.97 %			0.88 % ± 0.85 %	0.00 % ± 0.00 %

5 mM [U-$^{13}C_6$]glucose *iso. 2*

	Oleic acid-339	Stearic acid-341
M+1	1.54% ± 0.39%	2.65% ± 0.32%
M+2	3.87% ± 0.03%	0.09% ± 0.02%
M+3	0.00% ± 0.00%	0.00% ± 0.00%
M+4	0.03% ± 0.00%	0.00% ± 0.00%
M+5	0.01% ± 0.00%	0.01% ± 0.00%
M+6	0.00% ± 0.00%	0.01% ± 0.00%
M+7	0.00% ± 0.00%	0.00% ± 0.00%
M+8	0.00% ± 0.00%	0.01% ± 0.01%
M+9	0.00% ± 0.00%	0.00% ± 0.00%
M+10	0.01% ± 0.00%	0.00% ± 0.00%
M+11	0.00% ± 0.00%	0.00% ± 0.00%
M+12	0.02% ± 0.01%	0.06% ± 0.01%
M+13	0.01% ± 0.01%	0.02% ± 0.00%
M+14	0.06% ± 0.00%	0.04% ± 0.01%
M+15	0.02% ± 0.00%	0.02% ± 0.01%
M+16	0.04% ± 0.00%	0.01% ± 0.00%
M+17	0.01% ± 0.00%	0.00% ± 0.00%

M+18	0.01% ± 0.01%	0.00% ± 0.00%

Supplemental Tables S79 ^{13}C-Excess (mol%) of polar metabolites from [U-$^{13}C_6$]glucose DMEM Medium

^{13}C-Excess (mol%) of polar metabolites from DMEM medium for the 5 mM [U-$^{13}C_6$]glucose *immortal brown adipose tissue* labelling experiments. Mean and SD from three technical replicates are shown.

	con. 1	**iso. 1**	**con. 2**	**iso. 2**
Lactate-261	11.80 % ± 0.08 %	11.25 % ± 0.03 %	12.55% ± 0.15%	12.16% ± 0.07%
Glycolic acid-247	11.19 % ± 1.55 %	13.69 % ± 0.42 %	10.14% ± 2.40%	7.31% ± 2.63%
Ala-260	1.47 % ± 0.02 %	1.46 % ± 0.03 %	0.87% ± 0.03%	0.86% ± 0.04%
Gly-246	0.04 % ± 0.03 %	0.04 % ± 0.04 %	0.13% ± 0.08%	0.12% ± 0.11%
Oxalic acid-261		3.02 % ± 0.73 %	2.16% ± 0.70%	1.74% ± 1.15%
Val-288	0.11 % ± 0.00 %	0.09 % ± 0.02 %	0.07% ± 0.04%	0.09% ± 0.02%
Leu-274	0.31 % ± 0.03 %	0.34 % ± 0.02 %	0.29% ± 0.04%	0.25% ± 0.03%
Ile-274	0.58 % ± 0.05 %	0.57 % ± 0.03 %	0.51% ± 0.08%	0.53% ± 0.07%
Succinate-289	0.17 % ± 0.17 %	0.11 % ± 0.19 %	0.00% ± 0.00%	0.03% ± 0.02%
Glycerol-377	2.28 % ± 2.46 %	1.18 % ± 0.67 %	1.61% ± 0.96%	1.72% ± 0.86%
Met-320	0.40 % ± 0.01 %	0.32 % ± 0.02 %	0.45% ± 0.06%	0.35% ± 0.04%
Ser-390	0.38 % ± 0.06 %	0.32 % ± 0.08 %	0.33% ± 0.02%	0.31% ± 0.14%
Thr-404	0.04 % ± 0.03 %	0.11 % ± 0.09 %	0.10% ± 0.07%	0.06% ± 0.01%
Phe-336	1.06 % ± 0.04 %	1.17 % ± 0.05 %	1.20% ± 0.01%	1.34% ± 0.06%
Palmitic acid-313	0.03 % ± 0.01 %	0.03 % ± 0.01 %	0.02% ± 0.00%	0.03% ± 0.04%

Oleic acid-339	0.87 % ± 0.05 %	0.94 % ± 0.16 %	0.85% ± 0.01%	0.96% ± 0.20%
Stearic acid-341	0.45 % ± 0.09 %	0.44 % ± 0.03 %	0.39% ± 0.03%	0.37% ± 0.03%

Supplemental Table S80 Relative fractions of isotopologues (mol%) of polar metabolites from DMEM medium for the 10 mM [U-$^{13}C_6$]glucose *immortal brown adipose tissue* labelling experiments. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three technical replicates are shown.

5 mM [U-$^{13}C_6$]glucose *con. 1*					
	Ala-260	**Ile-274**	**Leu-274**	**Phe-336**	**Ser-390**
M+1	0.06% ± 0.07%	2.43% ± 0.37%	1.00% ± 0.16%	0.39% ± 0.20%	0.01% ± 0.01%
M+2	0.18% ± 0.02%	0.23% ± 0.08%	0.26% ± 0.01%	0.00% ± 0.00%	0.47% ± 0.18%
M+3	1.33% ± 0.02%	0.00% ± 0.00%	0.00% ± 0.00%	2.15% ± 0.04%	0.06% ± 0.05%
M+4		0.00% ± 0.00%	0.00% ± 0.00%	0.09% ± 0.02%	
M+5		0.00% ± 0.00%	0.01% ± 0.00%	0.46% ± 0.01%	
M+6				0.00% ± 0.01%	
M+7				0.00% ± 0.00%	
M+8				0.00% ± 0.00%	
M+9				0.00% ± 0.00%	

5 mM [U-$^{13}C_6$]glucose *con. 1*			
	Glycerol-377	**Glycolic acid-247**	**Lactate-261**
M+1	0.00% ± 0.00%	0.00 % ± 0.00 %	0.41 % ± 0.10 %
M+2	0.91% ± 1.57%	11.19 % ± 1.55 %	1.09 % ± 0.02 %

M+3	1.67% ± 1.55%		10.94 % ± 0.06 %

5 mM [U-$^{13}C_6$]glucose *con. 1*		
	Oleic acid-339	**Stearic acid-341**
M+1	1.01% ± 0.03%	1.65% ± 0.02%
M+2	3.74% ± 0.08%	0.04% ± 0.03%
M+3	0.00% ± 0.00%	0.00% ± 0.00%
M+4	0.05% ± 0.01%	0.06% ± 0.03%
M+5	0.01% ± 0.00%	0.00% ± 0.00%
M+6	0.02% ± 0.01%	0.03% ± 0.01%
M+7	0.01% ± 0.01%	0.01% ± 0.00%
M+8	0.02% ± 0.01%	0.02% ± 0.01%
M+9	0.01% ± 0.00%	0.01% ± 0.00%
M+10	0.01% ± 0.00%	0.03% ± 0.01%
M+11	0.00% ± 0.00%	0.01% ± 0.00%
M+12	0.02% ± 0.00%	0.10% ± 0.03%
M+13	0.01% ± 0.01%	0.03% ± 0.01%
M+14	0.06% ± 0.00%	0.12% ± 0.02%

M+15	0.03% ± 0.00%	0.03% ± 0.00%
M+16	0.17% ± 0.04%	0.06% ± 0.02%
M+17	0.03% ± 0.01%	0.01% ± 0.00%
M+18	0.08% ± 0.01%	0.02% ± 0.01%

5 mM [U-$^{13}C_6$]glucose *iso. 1*					
	Ala-260	**Ile-274**	**Leu-274**	**Phe-336**	**Ser-390**
M+1	0.02% ± 0.03%	2.45% ± 0.23%	1.13% ± 0.12%	0.33% ± 0.25%	0.03% ± 0.05%
M+2	0.13% ± 0.02%	0.20% ± 0.05%	0.26% ± 0.04%	0.00% ± 0.00%	0.38% ± 0.22%
M+3	1.36% ± 0.02%	0.00% ± 0.00%	0.00% ± 0.00%	2.25% ± 0.05%	0.05% ± 0.09%
M+4		0.00% ± 0.00%	0.00% ± 0.00%	0.10% ± 0.01%	
M+5		0.00% ± 0.00%	0.01% ± 0.00%	0.54% ± 0.02%	
M+6				0.05% ± 0.01%	
M+7				0.01% ± 0.01%	
M+8				0.00% ± 0.00%	
M+9				0.00% ± 0.00%	

5 mM [U-$^{13}C_6$]glucose *iso. 1*

	Glycerol-377	Glycolic acid-247	Lactate-261
M+1	0.02 % ± 0.04 %	0.00 % ± 0.00 %	0.46 % ± 0.13 %
M+2	0.42 % ± 0.37 %	13.69 % ± 0.42 %	0.90 % ± 0.03 %
M+3	0.89 % ± 0.43 %		10.50 % ± 0.02 %

5 mM [U-$^{13}C_6$]glucose *iso. 1*		
	Oleic acid-339	**Stearic acid-341**
M+1	1.69% ± 0.19%	1.43% ± 0.09%
M+2	3.72% ± 0.03%	0.05% ± 0.01%
M+3	0.00% ± 0.00%	0.00% ± 0.00%
M+4	0.06% ± 0.03%	0.16% ± 0.02%
M+5	0.01% ± 0.00%	0.01% ± 0.00%
M+6	0.02% ± 0.02%	0.03% ± 0.01%
M+7	0.01% ± 0.01%	0.01% ± 0.00%
M+8	0.01% ± 0.00%	0.02% ± 0.01%
M+9	0.01% ± 0.01%	0.01% ± 0.00%
M+10	0.01% ± 0.01%	0.05% ± 0.01%
M+11	0.00% ± 0.01%	0.02% ± 0.01%

M+12	0.02% ± 0.00%	0.06% ± 0.00%
M+13	0.03% ± 0.01%	0.03% ± 0.00%
M+14	0.06% ± 0.01%	0.11% ± 0.00%
M+15	0.02% ± 0.01%	0.03% ± 0.01%
M+16	0.18% ± 0.07%	0.06% ± 0.00%
M+17	0.04% ± 0.01%	0.01% ± 0.01%
M+18	0.09% ± 0.03%	0.02% ± 0.00%

5 mM [U-$^{13}C_6$]glucose *con. 2*					
	Ala-260	**Ile-274**	**Leu-274**	**Phe-336**	**Ser-390**
M+1	0.05% ± 0.05%	2.25% ± 0.48%	0.96% ± 0.10%	0.37% ± 0.26%	0.02% ± 0.04%
M+2	0.09% ± 0.04%	0.13% ± 0.09%	0.23% ± 0.07%	0.00% ± 0.00%	0.40% ± 0.07%
M+3	0.79% ± 0.02%	0.00% ± 0.00%	0.00% ± 0.00%	2.45% ± 0.07%	0.05% ± 0.04%
M+4		0.00% ± 0.00%	0.00% ± 0.00%	0.11% ± 0.01%	
M+5		0.00% ± 0.00%	0.01% ± 0.01%	0.52% ± 0.02%	
M+6				0.01% ± 0.01%	
M+7				0.00% ± 0.00%	

M+8				0.00% ± 0.00%	
M+9				0.00% ± 0.00%	

5 mM [U-$^{13}C_6$]glucose *con. 2*			
	Glycerol-377	**Glycolic acid-247**	**Lactate-261**
M+1	0.33 % ± 0.57 %	0.00 % ± 0.00 %	0.29 % ± 0.06 %
M+2	0.78 % ± 0.53 %	10.14 % ± 2.40 %	1.01 % ± 0.01 %
M+3	0.98 % ± 0.87 %		11.78 % ± 0.13 %

5 mM [U-$^{13}C_6$]glucose *con. 2*		
	Oleic acid-339	**Stearic acid-341**
M+1	1.18% ± 0.03%	1.30% ± 0.18%
M+2	3.79% ± 0.06%	0.03% ± 0.02%
M+3	0.00% ± 0.00%	0.00% ± 0.00%
M+4	0.04% ± 0.01%	0.10% ± 0.01%
M+5	0.01% ± 0.00%	0.01% ± 0.01%
M+6	0.01% ± 0.01%	0.03% ± 0.01%
M+7	0.01% ± 0.01%	0.01% ± 0.01%

M+8	0.01% ± 0.00%	0.01% ± 0.01%
M+9	0.00% ± 0.00%	0.01% ± 0.00%
M+10	0.00% ± 0.00%	0.03% ± 0.01%
M+11	0.00% ± 0.00%	0.02% ± 0.00%
M+12	0.02% ± 0.00%	0.06% ± 0.00%
M+13	0.01% ± 0.01%	0.02% ± 0.00%
M+14	0.05% ± 0.00%	0.10% ± 0.01%
M+15	0.03% ± 0.00%	0.04% ± 0.00%
M+16	0.15% ± 0.01%	0.07% ± 0.01%
M+17	0.03% ± 0.00%	0.01% ± 0.00%
M+18	0.09% ± 0.01%	0.01% ± 0.00%

5 mM [U-$^{13}C_6$]glucose *iso. 2*					
	Ala-260	**Ile-274**	**Leu-274**	**Phe-336**	**Ser-390**
M+1	0.06% ± 0.08%	2.26% ± 0.42%	0.76% ± 0.11%	0.05% ± 0.06%	0.00% ± 0.00%
M+2	0.17% ± 0.03%	0.19% ± 0.02%	0.23% ± 0.03%	0.00% ± 0.00%	0.37% ± 0.18%

M+3	0.72% ± 0.01%	0.00% ± 0.00%	0.00% ± 0.00%	2.61% ± 0.09%	0.07% ± 0.06%
M+4		0.00% ± 0.00%	0.00% ± 0.00%	0.12% ± 0.03%	
M+5		0.00% ± 0.00%	0.01% ± 0.01%	0.65% ± 0.08%	
M+6				0.06% ± 0.01%	
M+7				0.01% ± 0.01%	
M+8				0.00% ± 0.00%	
M+9				0.00% ± 0.00%	

5 mM [U-$^{13}C_6$]glucose *iso. 2*			
	Glycerol-377	**Glycolic acid-247**	**Lactate-261**
M+1	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.40 % ± 0.12 %
M+2	0.20 % ± 0.22 %	7.31 % ± 2.63 %	0.97 % ± 0.01 %
M+3	1.59 % ± 0.78 %		11.38 % ± 0.04 %

5 mM [U-$^{13}C_6$]glucose *iso. 2*		
	Oleic acid-339	**Stearic acid-341**
M+1	1.42% ± 0.34%	1.36% ± 0.15%
M+2	3.83% ± 0.10%	0.02% ± 0.04%

M+3	0.00% ± 0.00%	0.00% ± 0.00%
M+4	0.07% ± 0.05%	0.11% ± 0.01%
M+5	0.01% ± 0.00%	0.01% ± 0.00%
M+6	0.03% ± 0.02%	0.01% ± 0.00%
M+7	0.01% ± 0.01%	0.01% ± 0.00%
M+8	0.01% ± 0.01%	0.01% ± 0.00%
M+9	0.01% ± 0.01%	0.01% ± 0.01%
M+10	0.02% ± 0.01%	0.04% ± 0.01%
M+11	0.00% ± 0.01%	0.01% ± 0.00%
M+12	0.02% ± 0.01%	0.07% ± 0.00%
M+13	0.02% ± 0.01%	0.04% ± 0.00%
M+14	0.07% ± 0.03%	0.09% ± 0.00%
M+15	0.03% ± 0.00%	0.04% ± 0.00%
M+16	0.20% ± 0.09%	0.04% ± 0.00%
M+17	0.04% ± 0.02%	0.00% ± 0.00%
M+18	0.08% ± 0.04%	0.01% ± 0.00%

Supplemental Tables S81 Quantitative analysis of polar metabolites from [U-$^{13}C_6$]glucose labelled *immortal brown adipose tissue (IBAT)*

Quantitative analysis (µmol) per welle of polar metabolites from experiments with *immortal brown adipose tissue* grown in DMEM medium supplemented with 5 mM [U-$^{13}C_6$]glucose. Mean and SD from three technical replicates are shown.

[µmol]	con. 1	iso. 1	con. 2	iso. 2
Lactate	0.042086 ± 0.003433	0.075668 ± 0.008680	0.035933 ± 0.004274	0.038492 ± 0.000669
Ala	0.018543 ± 0.003980	0.018030 ± 0.002784	0.010409 ± 0.001474	0.010361 ± 0.000322
Gly	0.011849 ± 0.002409	0.016290 ± 0.002034	0.002485 ± 0.000641	0.003074 ± 0.000152
Val	0.016864 ± 0.003584	0.020423 ± 0.003095	0.008810 ± 0.001143	0.011632 ± 0.000845
Leu	0.020048 ± 0.004089	0.023010 ± 0.003267	0.009219 ± 0.000882	0.014562 ± 0.001079
Ile	0.086898 ± 0.016118	0.087784 ± 0.013729	0.051376 ± 0.010354	0.053751 ± 0.009967
Glycerol	0.008277 ± 0.001198	0.051707 ± 0.029772	0.009114 ± 0.000384	0.011136 ± 0.000554
Met	0.003159 ± 0.000808	0.003737 ± 0.000738	0.001423 ± 0.000200	0.001530 ± 0.000078
Ser	0.006858 ± 0.001696	0.009844 ± 0.002282	0.001174 ± 0.000111	0.001859 ± 0.000064
Thr	0.035784 ± 0.009359	0.048153 ± 0.010490	0.005133 ± 0.000168	0.006614 ± 0.000714
Phe	0.004101 ± 0.000757	0.014589 ± 0.016185	0.003978 ± 0.001876	0.005616 ± 0.001580
Palmitic acid	4.795884 ± 0.454051	5.304307 ± 0.349064	3.167714 ± 0.264646	4.249008 ± 0.337963
Oleic acid	0.073141 ± 0.033675	0.218404 ± 0.040976	0.079567 ± 0.027675	0.110017 ± 0.035039
Stearic acid	1.542828 ± 1.108264	2.025593 ± 0.975586	0.779835 ± 0.326060	0.733958 ± 0.249203

Supplemental Tables S82 Quantitative analysis of polar metabolites from [U-$^{13}C_6$]glucose DMEM Medium

Quantitative analysis (µmol) per welle of polar metabolites from DMEM medium for the 5 mM [U-$^{13}C_6$]glucose *immortal brown adipose tissue* labelling experiments. Mean and SD from three technical replicates are shown.

[µmol]	con. 1	iso. 1	con. 2	iso. 2
Lactate	30.550893 ± 18.64994	194.6612 ± 14.28916	16.86387 ± 0.949312	139.4159 ± 7.088758
Ala	1.839334248 ± 1.111758	6.735641 ± 0.33522	3.498223 ± 0.126291	10.97667 ± 0.367504
Gly	0.642631667 ± 0.383898	2.83704 ± 0.156176	0.569796 ± 0.008946	1.346454 ± 0.074585
Val	22.02170808 ± 13.38471	95.09239 ± 5.6325	12.11178 ± 0.658165	81.03297 ± 4.132642
Leu	26.51126842 ± 22.70684	114.276 ± 8.241424	14.67447 ± 0.523629	107.4675 ± 4.845047
Ile	28.55199218 ± 16.83437	109.6208 ± 7.168266	14.31771 ± 0.430753	101.4939 ± 5.112431
Met	5.056538581 ± 3.005256	9.864721 ± 0.149131	1.364524 ± 0.081981	9.835348 ± 0.85823
Ser	1.686837992 ± 0.903873	5.237292 ± 0.081887	1.190628 ± 0.051437	3.555665 ± 0.204101
Thr	3.61920197 ± 2.720387	13.9299 ± 1.422987	2.636446 ± 0.058084	11.1532 ± 0.577926
Phe	5.553554747 ± 4.275415	19.83453 ± 0.32214	4.193164 ± 0.325752	19.884 ± 2.161104
Palmitic acid	150.9803818 ± 22.24874	354.436 ± 71.55205	76.31805 ± 13.11853	271.5586 ± 63.57267
Oleic acid	10.88892806 ± 1.892132	25.66312 ± 1.624252	6.266155 ± 0.637701	16.56762 ± 1.519596
Stearic acid	48.2126498 ± 20.64512	79.01293 ± 29.26341	29.42802 ± 14.35785	57.65936 ± 23.18967

Supplemental Table S83 ^{13}C-Excess (mol%) of glycogen-bound glucose from [U-$^{13}C_6$]glucose labelled *immortal brown adipose tissue (IBAT)*

^{13}C-Excess (mol%) of glycogen-bound glucose from experiments with *immortal brown adipose tissue* grown in DMEM medium supplemented with 5 mM [U-$^{13}C_6$]glucose. Mean and SD from three technical replicates are shown.

	con. 1	**iso. 1**	**con. 2**	**iso. 2**
Glc-287	0.97% ± 0.13%	2.70% ± 0.20%	2.03% ± 0.13%	3.21% ± 0.18%

Supplemental Table S84 Relative fractions of isotopologues (mol%) of glycogen-bound glucose from experiments with *immortal brown adipose tissue* grown in DMEM medium supplemented with 5 mM [U-$^{13}C_6$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three technical replicates are shown.

Glc-287	**con. 1**	**iso. 1**	**con. 2**	**iso. 2**
M+1	0.00% ± 0.00%	0.04% ± 0.04%	0.02% ± 0.03%	0.00% ± 0.00%
M+2	0.02% ± 0.02%	0.03% ± 0.06%	0.00% ± 0.00%	0.01% ± 0.02%
M+3	0.12% ± 0.03%	0.00% ± 0.01%	0.07% ± 0.06%	0.01% ± 0.02%
M+4	0.02% ± 0.03%	0.03% ± 0.03%	0.03% ± 0.01%	0.02% ± 0.02%
M+5	0.05% ± 0.09%	0.06% ± 0.07%	0.06% ± 0.00%	0.09% ± 0.04%
M+6	0.85% ± 0.15%	2.60% ± 0.20%	1.91% ± 0.17%	3.11% ± 0.17%

Supplemental Table S85 ^{13}C-Excess (mol%) of free sugars from [U-$^{13}C_6$]glucose medium

^{13}C-Excess (mol%) of free glucose from DMEM medium for the 5 mM [U-$^{13}C_6$]glucose *immortal brown adipose tissue* labelling experiments. Mean and SD from three technical replicates are shown.

	con. 1	**iso. 1**	**con. 2**	**iso. 2**
Glc-287	15.45% ± 1.01%	14.51% ± 1.03%	14.64% ± 0.75%	14.58% ± 1.23%

Supplemental Table S86 Relative fractions of isotopologues (mol%) of free glucose from DMEM medium for the 5 mM [U-$^{13}C_6$]glucose *immortal brown adipose tissue* labelling experiments. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three technical replicates are shown.

Glc-287	**con. 1**	**iso. 1**	**con. 2**	**iso. 2**
M+1	0.04% ± 0.08%	0.11% ± 0.13%	0.17% ± 0.29%	0.15% ± 0.21%
M+2	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+3	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+4	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+5	0.44% ± 0.02%	0.42% ± 0.01%	0.42% ± 0.01%	0.42% ± 0.03%
M+6	15.07% ± 0.98%	14.14% ± 1.01%	14.26% ± 0.72%	14.21% ± 1.19%

Supplemental Table S87 ^{13}C-Excess (mol%) of lipid-bound fatty acids from [U-$^{13}C_6$]glucose labelled *immortal brown adipose tissue (IBAT)*

^{13}C-Excess (mol%) of lipid-bound fatty acids from experiments with *immortal brown adipose tissue* grown in DMEM medium supplemented with 10 mM [U-$^{13}C_6$]glucose. Mean and SD from three technical replicates are shown.

	con. 1	**iso. 1**	**con. 2**	**iso. 2**
Z-11-tetradecenoic acid-240	0.38% ± 0.12%	0.38% ± 0.13%	0.40% ± 0.04%	0.93% ± 0.53%
Tetradecanoic acid-242	0.40% ± 0.13%	0.42% ± 0.10%	0.41% ± 0.09%	0.40% ± 0.19%
Pentadecanoic acid-256	0.23% ± 0.11%	0.23% ± 0.06%	0.25% ± 0.06%	0.22% ± 0.11%
9-Hexadecenoic acid-268	0.41% ± 0.14%	0.44% ± 0.15%	0.39% ± 0.15%	0.38% ± 0.14%
Hexadecanoic acid-270	0.31% ± 0.08%	0.35% ± 0.03%	0.33% ± 0.02%	0.30% ± 0.09%
Heptadecanoic acid-284	0.12% ± 0.04%	0.11% ± 0.04%	0.05% ± 0.02%	0.09% ± 0.04%
6-Octadecenoic acid-296	0.27% ± 0.13%	0.27% ± 0.13%	0.27% ± 0.08%	0.32% ± 0.13%
9-Octadecenoic acid-296	0.44% ± 0.17%	0.29% ± 0.11%	0.30% ± 0.03%	0.31% ± 0.06%
Octadecanoic acid-298	0.13% ± 0.07%	0.12% ± 0.07%	0.10% ± 0.06%	0.10% ± 0.05%

Supplemental Table S88 Relative fractions of isotopologues (mol%) of lipid-bound fatty acids from experiments with immortal brown adipose tissue grown in DMEM medium supplemented with 5 mM [U-$^{13}C_6$]glucose. M+x represents the mass of the unlabelled metabolite plus x labelled ^{13}C-atoms. Mean and SD from three technical replicates are shown.

5 mM [U-$^{13}C_6$]glucose *con. 1*					
	Z-11-tetradecenoic acid-240	**Tetradecanoic acid-242**	**Pentadecanoic acid-256**	**9-Hexadecenoic acid-268**	**Hexadecanoic acid-270**
M+1	3.03 % ± 1.63 %	4.98 % ± 1.79 %	2.92 % ± 1.70 %	6.16 % ± 2.63 %	4.55 % ± 1.15%
M+2	0.14 % ± 0.12 %	0.13 % ± 0.03 %	0.06 % ± 0.03 %	0.00 % ± 0.00 %	0.05 % ± 0.04%
M+3	0.02 % ± 0.04 %	0.00 % ± 0.00 %	0.00 % ± 0.01 %	0.00 % ± 0.00 %	0.00 % ± 0.00%
M+4	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00%
M+5	0.04 % ± 0.07 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.01 % ± 0.02 %	0.00 % ± 0.00%
M+6	0.05 % ± 0.06 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.01 %	0.00 % ± 0.00%
M+7	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.01 % ± 0.01 %	0.00 % ± 0.00 %	0.00 % ± 0.00%
M+8	0.00 % ± 0.00 %	0.01 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00%
M+9	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00%

M+10	0.01 %	±	0.02 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00%
M+11	0.08 %	±	0.08 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.05 %	±	0.04 %	0.00 %	±	0.00%
M+12	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00%
M+13	0.00 %	±	0.00 %	0.01 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00%
M+14	0.05 %	±	0.09 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00%
M+15	0.00 %	±	0.00 %	0.04 %	±	0.02 %	0.03 %	±	0.01 %	0.01 %	±	0.02 %	0.03 %	±	0.01%
M+16							0.00 %	±	0.01 %	0.00 %	±	0.00 %	0.00 %	±	0.00%
M+17										0.00 %	±	0.00 %	0.00 %	±	0.00%

5 mM [U-$^{13}C_6$]glucose *con. 1*												
	Heptadecanoic acid-284			**6-Octadecenoic acid-296**			**9-Octadecenoic acid-296**			**Octadecanoic acid-298**		
M+1	1.88%	±	0.75%	4.71%	±	2.61%	3.00%	±	1.91%	2.11%	±	1.29%
M+2	0.00%	±	0.00%	0.10%	±	0.05%	0.12%	±	0.09%	0.00%	±	0.00%
M+3	0.01%	±	0.01%	0.00%	±	0.00%	0.00%	±	0.00%	0.00%	±	0.00%
M+4	0.00%	±	0.00%	0.00%	±	0.00%	0.02%	±	0.03%	0.00%	±	0.00%

M+5	0.00% ± 0.00%	0.00% ± 0.00%	0.02% ± 0.02%	0.00% ± 0.00%
M+6	0.00% ± 0.00%	0.01% ± 0.02%	0.00% ± 0.01%	0.00% ± 0.00%
M+7	0.00% ± 0.00%	0.00% ± 0.00%	0.02% ± 0.03%	0.01% ± 0.01%
M+8	0.00% ± 0.00%	0.01% ± 0.01%	0.07% ± 0.11%	0.00% ± 0.01%
M+9	0.01% ± 0.00%	0.01% ± 0.02%	0.09% ± 0.05%	0.01% ± 0.01%
M+10	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+11	0.00% ± 0.00%	0.00% ± 0.00%	0.02% ± 0.03%	0.00% ± 0.00%
M+12	0.01% ± 0.00%	0.00% ± 0.00%	0.03% ± 0.04%	0.00% ± 0.00%
M+13	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.01%	0.00% ± 0.00%
M+14	0.00% ± 0.00%	0.00% ± 0.00%	0.03% ± 0.03%	0.00% ± 0.00%
M+15	0.01% ± 0.00%	0.00% ± 0.00%	0.09% ± 0.05%	0.00% ± 0.01%
M+16	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+17	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+18	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+19		0.00% ± 0.00%	0.05% ± 0.09%	0.00% ± 0.00%

5 mM [U-$^{13}C_6$]glucose *iso. 1*					
	Z-11-tetradecenoic acid-240	**Tetradecanoic acid-242**	**Pentadecanoic acid-256**	**9-Hexadecenoic acid-268**	**Hexadecanoic acid-270**

M+1	2.96 % ± 1.36 %	5.20 % ± 1.38 %	3.16 % ± 1.02 %	6.54 % ± 1.95 %	5.15 % ± 0.51%
M+2	0.16 % ± 0.18 %	0.10 % ± 0.02 %	0.04 % ± 0.04 %	0.01 % ± 0.01 %	0.07 % ± 0.07%
M+3	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00%
M+4	0.07 % ± 0.08 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00%
M+5	0.10 % ± 0.07 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.02 % ± 0.03 %	0.00 % ± 0.00%
M+6	0.02 % ± 0.03 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.01 % ± 0.01 %	0.00 % ± 0.00%
M+7	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00%
M+8	0.01 % ± 0.01 %	0.01 % ± 0.00 %	0.00 % ± 0.01 %	0.01 % ± 0.01 %	0.00 % ± 0.00%
M+9	0.04 % ± 0.04 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00%
M+10	0.03 % ± 0.02 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00%
M+11	0.05 % ± 0.06 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.05 % ± 0.04 %	0.00 % ± 0.00%
M+12	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.01 % ± 0.01 %	0.00 % ± 0.00%
M+13	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00%

	%		%	%		%	%		%	%		%
M+14	0.01 %	±	0.02 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00%
M+15	0.00 %	±	0.00 %	0.05 %	±	0.01 %	0.03 %	±	0.01 %	0.00 %	±	0.00 %	0.04 %	±	0.00%
M+16							0.00 %	±	0.00 %	0.00 %	±	0.01 %	0.00 %	±	0.00%
M+17										0.00 %	±	0.00 %	0.00 %	±	0.00%

5 mM [U-$^{13}C_6$]glucose *iso. 1*												
	Heptadecanoic acid-284			**6-Octadecenoic acid-296**			**9-Octadecenoic acid-296**			**Octadecanoic acid-298**		
M+1	0.59%	±	0.51%	4.21%	±	1.89%	2.93%	±	1.79%	1.93%	±	0.85%
M+2	0.01%	±	0.01%	0.11%	±	0.06%	0.14%	±	0.11%	0.00%	±	0.00%
M+3	0.01%	±	0.02%	0.00%	±	0.00%	0.02%	±	0.04%	0.00%	±	0.00%
M+4	0.01%	±	0.01%	0.00%	±	0.00%	0.00%	±	0.00%	0.00%	±	0.00%
M+5	0.00%	±	0.00%	0.01%	±	0.02%	0.04%	±	0.02%	0.00%	±	0.00%
M+6	0.02%	±	0.02%	0.00%	±	0.00%	0.06%	±	0.07%	0.00%	±	0.00%
M+7	0.01%	±	0.01%	0.02%	±	0.03%	0.01%	±	0.01%	0.00%	±	0.01%
M+8	0.01%	±	0.01%	0.01%	±	0.01%	0.02%	±	0.02%	0.00%	±	0.00%
M+9	0.02%	±	0.02%	0.00%	±	0.00%	0.03%	±	0.06%	0.00%	±	0.00%

M+10	0.01% ± 0.01%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+11	0.00% ± 0.00%	0.01% ± 0.01%	0.02% ± 0.04%	0.00% ± 0.00%
M+12	0.01% ± 0.01%	0.00% ± 0.00%	0.03% ± 0.05%	0.00% ± 0.00%
M+13	0.01% ± 0.01%	0.00% ± 0.00%	0.01% ± 0.01%	0.00% ± 0.00%
M+14	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+15	0.01% ± 0.01%	0.00% ± 0.00%	0.01% ± 0.02%	0.01% ± 0.02%
M+16	0.02% ± 0.02%	0.02% ± 0.02%	0.02% ± 0.03%	0.00% ± 0.01%
M+17	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+18	0.01% ± 0.01%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+19		0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%

5 mM [U-$^{13}C_6$]glucose *con. 2*					
	Z-11-tetradecenoic acid-240	**Tetradecanoic acid-242**	**Pentadecanoic acid-256**	**9-Hexadecenoic acid-268**	**Hexadecanoic acid-270**
M+1	2.88 % ± 1.28 %	5.23 % ± 1.19 %	3.36 % ± 0.94 %	5.56 % ± 1.77 %	4.83 % ± 0.21%
M+2	0.24 % ± 0.09 %	0.12 % ± 0.03 %	0.03 % ± 0.03 %	0.00 % ± 0.00 %	0.05 % ± 0.05%
M+3	0.01 % ± 0.01 %	0.00 % ± 0.01 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00%
M+4	0.06 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00%

	%		%	%		%	%		%	%		
M+5	0.01 %	±	0.02 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.01 %	0.00 %	±	0.00%
M+6	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00%
M+7	0.06 %	±	0.11 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.01 %	±	0.00 %	0.00 %	±	0.00%
M+8	0.02 %	±	0.03 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.02 %	±	0.02 %	0.00 %	±	0.00%
M+9	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.01 %	±	0.01 %	0.00 %	±	0.00%
M+10	0.09 %	±	0.16 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00%
M+11	0.03 %	±	0.05 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.06 %	±	0.05 %	0.00 %	±	0.00%
M+12	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.01 %	±	0.02 %	0.00 %	±	0.00%
M+13	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00%
M+14	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00%
M+15	0.03 %	±	0.05 %	0.04 %	±	0.01 %	0.02 %	±	0.01 %	0.00 %	±	0.00 %	0.04 %	±	0.00%
M+16							0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00%

M+17				0.00 % ± 0.00 %	0.00 % ± 0.00%

5 mM [U-$^{13}C_6$]glucose *con. 2*				
	Heptadecanoic acid-284	**6-Octadecenoic acid-296**	**9-Octadecenoic acid-296**	**Octadecanoic acid-298**
M+1	0.41% ± 0.39%	4.45% ± 1.50%	3.19% ± 1.63%	1.51% ± 0.77%
M+2	0.01% ± 0.01%	0.10% ± 0.03%	0.21% ± 0.13%	0.00% ± 0.01%
M+3	0.02% ± 0.03%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+4	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+5	0.00% ± 0.00%	0.01% ± 0.02%	0.03% ± 0.02%	0.00% ± 0.00%
M+6	0.01% ± 0.01%	0.00% ± 0.00%	0.03% ± 0.05%	0.00% ± 0.00%
M+7	0.00% ± 0.00%	0.01% ± 0.01%	0.03% ± 0.02%	0.00% ± 0.01%
M+8	0.01% ± 0.00%	0.02% ± 0.02%	0.00% ± 0.00%	0.00% ± 0.00%
M+9	0.00% ± 0.00%	0.01% ± 0.01%	0.02% ± 0.04%	0.00% ± 0.00%
M+10	0.01% ± 0.01%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+11	0.00% ± 0.00%	0.00% ± 0.00%	0.03% ± 0.04%	0.00% ± 0.00%
M+12	0.00% ± 0.00%	0.00% ± 0.00%	0.05% ± 0.08%	0.00% ± 0.00%
M+13	0.01% ± 0.01%	0.00% ± 0.00%	0.03% ± 0.05%	0.00% ± 0.00%
M+14	0.01% ± 0.01%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%

M+15	0.00% ± 0.00%	0.01% ± 0.02%	0.00% ± 0.01%	0.00% ± 0.01%
M+16	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+17	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%	0.01% ± 0.01%
M+18	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+19		0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%

5 mM [U-$^{13}C_6$]glucose *iso. 2*					
	Z-11-tetradecenoic acid-240	**Tetradecanoic acid-242**	**Pentadecanoic acid-256**	**9-Hexadecenoic acid-268**	**Hexadecanoic acid-270**
M+1	2.80 % ± 1.87 %	5.18 % ± 2.27 %	2.91 % ± 1.69 %	4.85 % ± 0.85 %	4.41 % ± 1.35%
M+2	0.12 % ± 0.17 %	0.11 % ± 0.05 %	0.07 % ± 0.01 %	0.00 % ± 0.00 %	0.05 % ± 0.08%
M+3	0.10 % ± 0.12 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00%
M+4	0.06 % ± 0.06 %	0.00 % ± 0.00 %	0.00 % ± 0.01 %	0.03 % ± 0.05 %	0.00 % ± 0.00%
M+5	0.19 % ± 0.20 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00%
M+6	0.05 % ± 0.05 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.02 % ± 0.03 %	0.00 % ± 0.00%
M+7	0.25 % ± 0.21 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00%

M+8	0.11 %	±	0.17 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.01 %	±	0.01 %	0.00 %	±	0.00%
M+9	0.03 %	±	0.06 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.01 %	0.00 %	±	0.00%
M+10	0.11 %	±	0.15 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.01 %	±	0.02 %	0.00 %	±	0.00%
M+11	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.10 %	±	0.10 %	0.00 %	±	0.00%
M+12	0.35 %	±	0.60 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00%
M+13	0.04 %	±	0.07 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00%
M+14	0.02 %	±	0.03 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00%
M+15	0.00 %	±	0.00 %	0.04 %	±	0.03 %	0.02 %	±	0.01 %	0.00 %	±	0.00 %	0.04 %	±	0.01%
M+16							0.00 %	±	0.00 %	0.00 %	±	0.00 %	0.00 %	±	0.00%
M+17										0.00 %	±	0.00 %	0.00 %	±	0.00%

5 mM [U-$^{13}C_6$]glucose *iso. 2*				
	Heptadecanoic acid-284	**6-Octadecenoic acid-296**	**9-Octadecenoic acid-296**	**Octadecanoic acid-298**
M+1	0.70% ± 0.94%	4.75% ± 2.59%	3.10% ± 2.23%	1.48% ± 0.89%

M+2	0.00% ± 0.01%	0.06% ± 0.05%	0.19% ± 0.16%	0.01% ± 0.02%
M+3	0.02% ± 0.02%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+4	0.00% ± 0.01%	0.00% ± 0.00%	0.01% ± 0.01%	0.00% ± 0.00%
M+5	0.00% ± 0.01%	0.01% ± 0.01%	0.02% ± 0.03%	0.00% ± 0.00%
M+6	0.01% ± 0.01%	0.01% ± 0.01%	0.06% ± 0.08%	0.00% ± 0.00%
M+7	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%	0.01% ± 0.01%
M+8	0.00% ± 0.01%	0.01% ± 0.01%	0.02% ± 0.03%	0.00% ± 0.00%
M+9	0.01% ± 0.00%	0.03% ± 0.02%	0.00% ± 0.01%	0.00% ± 0.00%
M+10	0.01% ± 0.01%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+11	0.00% ± 0.00%	0.00% ± 0.00%	0.04% ± 0.06%	0.00% ± 0.00%
M+12	0.01% ± 0.01%	0.02% ± 0.02%	0.01% ± 0.02%	0.00% ± 0.00%
M+13	0.00% ± 0.00%	0.00% ± 0.00%	0.01% ± 0.02%	0.00% ± 0.00%
M+14	0.00% ± 0.00%	0.02% ± 0.02%	0.04% ± 0.06%	0.00% ± 0.00%
M+15	0.00% ± 0.00%	0.00% ± 0.00%	0.01% ± 0.01%	0.00% ± 0.01%
M+16	0.01% ± 0.01%	0.01% ± 0.01%	0.02% ± 0.03%	0.01% ± 0.01%
M+17	0.02% ± 0.02%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+18	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%	0.00% ± 0.00%
M+19	±	0.00% ± 0.00%	0.00% ± 0.00%	0.01% ± 0.01%

Supplemental Table S89 Composition of DMEM(5030)-medium

	D5030
	[powder]
COMPONENT	g/L
Inorganic Salts	
$CaCl_2$	0.2
$Fe(NO_3)_3 \cdot 9H2O$	0.0001
$MgSO_4$	0.09767
KCl	0.4
$NaHCO_3$	—
NaCl	6.4
NaH_2PO_4	0.109
Amino Acids	
L-Alanyl-L-Glutamine	—
L-Arginine • HCl	0.084
L-Cystine • 2HCl	0.0626
L-Glutamine	—
Glycine	0.03
L-Histidine • HCl • H2O	0.042
L-Isoleucine	0.105
L-Leucine	0.105
L-Lysine • HCl	0.146
L-Methionine	0.03
L-Phenylalanine	0.066
L-Serine	0.042
L-Threonine	0.095
L-Tryptophan	0.016
L-Tyrosine	0.10379

• 2Na • 2H2O	
L-Tyrosine	—
L-Valine	0.094
Vitamins	
Choline Chloride	0.004
Folic Acid	0.004
myo-Inositol	0.0072
Niacinamide	0.004
D-Pantothenic Acid • ½Ca	0.004
Pyridoxal • HCl	0.004
Pyridoxine • HCl	—
Riboflavin	0.0004
Thiamine • HCl	0.004
Other	
D-Glucose	—
HEPES	—
Phenol Red • Na	—
Pyruvic Acid • Na	—
ADD	
Glucose	1.0
L-Glutamine	0.584
$NaHCO_3$	3.7

Supplemental Tables S90 D-Excess (mol%) of protein-bound amino acids from myo-Inositol-C-d_6 labelling experiment

D-Excess (mol%) of protein-bound amino acids from experiments with *F.* W12-1067 WT and Δmyo-inositol mutant grown in CDM medium supplemented with 9.13 mM myo-Inositol-C-d_6. Mean and SD from one experiment are shown.

	WT	Δmyo-inositol mutant
Ala	0.62% ± 0.02%	0.14% ± 0.00%
Asp	0.38% ± 0.07%	0.23% ± 0.01%
Glu	0.67% ± 0.05%	0.28% ± 0.15%
Gly	0.06% ± 0.02%	0.17% ± 0.15%
His	2.08% ± 0.08%	1.23% ± 0.09%
Ile	0.02% ± 0.01%	0.02% ± 0.03%
Leu	0.02% ± 0.01%	0.07% ± 0.00%
Lys	0.08% ± 0.01%	0.11% ± 0.05%
Met	0.12% ± 0.09%	0.10% ± 0.03%
Phe	5.29% ± 0.03%	0.08% ± 0.02%
Pro	0.04% ± 0.01%	0.03% ± 0.01%
Ser	0.03% ± 0.00%	0.08% ± 0.07%
Thr	0.11% ± 0.05%	0.13% ± 0.15%
Tyr	0.75% ± 0.09%	0.06% ± 0.04%
Val	0.03% ± 0.03%	0.05% ± 0.03%

Supplemental Table S91 D-Excess (mol%) of polar metabolites from myo-Inositol-C-d_6 labelling experiment

D-Excess (mol%) of polar metabolites from experiments with *F.* W12-1067 WT and Δmyo-inositol mutant grown in CDM medium supplemented with 9.13 mM myo-Inositol-C-d_6. Mean and SD from two independent experiments are shown.

	WT	Δmyo-inositol mutant
Lactate	3.14% ± 0.00%	0.72% ± 0.02%
Glycolic acid	1.70% ± 0.17%	0.76% ± 0.16%
Alanine	0.77% ± 0.01%	0.18% ± 0.02%
Glycine	0.35% ± 0.01%	0.25% ± 0.02%
3-hydroxy butyric acid		0.09 % ± 0.08 %
Valine	0.01% ± 0.00%	0.05% ± 0.02%
leucine	0.01% ± 0.01%	0.06% ± 0.02%
Isoleucine	0.01% ± 0.01%	0.05% ± 0.02%

Succinic acid	1.83%	±	0.04%			
Fumaric acid	0.63%	±	0.05%	0.24%	±	0.12%
Glycerol				0.17%	±	0.10%
Methionine	0.09%	±	0.09%	0.12%	±	0.09%
Serine	0.02%	±	0.01%	0.04%	±	0.03%
Threonine	0.20%	±	0.11%	0.18%	±	0.16%
Phenylalanine	8.21%	±	0.06%			
Malic acid	0.55%	±	0.20%	0.13%	±	0.14%
Aspartate	0.14%	±	0.05%	0.39%	±	0.04%
Glutamate	1.05%	±	0.07%	0.23 %	±	0.06 %
Palmitic acid	0.93 %	±	0.34 %	0.33 %	±	0.06 %
Stearic acid	1.26 %	±	0.46 %	0.28%	±	0.03%
Citric acid				0.03%	±	0.03%

Supplemental Table S92 D-Excess (mol%) of sugars from myo-Inositol-C-d_6 labelling experiment

D-Excess (mol%) of sugars from experiments with *F.* W12-1067 WT and Δmyo-inositol mutant grown in CDM medium supplemented with 9.13 mM myo-Inositol-C-d_6. Mean and SD from two independent experiments are shown.

	WT			Δmyo-inositol mutant		
Glucose in glycogen	4.63 %	±	0.41 %	0.17 %	±	0.07 %
Glucosamine						
Muramic acid						

Supplemental Table S93 Relative fractions of isotopologues (mol%) of amino acids from experiments with *F.* W12-1067 WT and Δmyo-inositol mutant grown in CDM medium supplemented with 9.13 mM myo-Inositol-C-d_6. M+x represents the mass M of the unlabelled metabolite plus x labelled D-atoms. Mean and SD from one experiment are shown.

9.13 mM myo-Inositol-C-d_6 *F.* W12-1067 WT					
	Alanine	Glutamate	Histidine	Phenylalanine	Tyrosine
M+1	2.08 % ± 0.08 %	3.25 % ± 0.96 %	11.25 ± 0.42 % %	21.24 ± 0.13 % %	3.03 % ± 0.59 %

M+2	0.51 % ± 0.02 %	0.39 % ± 0.34 %	0.04 % ± 0.04 %	8.12 % ± 0.04 %	1.05 % ± 0.11 %
M+3	0.00 % ± 0.00 %	0.00 % ± 0.01 %	0.36 % ± 0.07 %	2.66 % ± 0.01 %	0.25 % ± 0.01 %
M+4	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.01 % ± 0.01 %	0.38 % ± 0.01 %	0.03 % ± 0.01 %
M+5	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.01 % ± 0.01 %	0.03 % ± 0.01 %	0.00 % ± 0.00 %
M+6		0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %	0.00 % ± 0.01 %
M+7				0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+8				0.05 % ± 0.00 %	0.00 % ± 0.00 %
M+9				0.01 % ± 0.00 %	

9.13 mM myo-Inositol-C-d_6 *F.* W12-1067 Δmyo-inositol mutant	
	Histidine
M+1	6.70 % ± 0.38 %
M+2	0.08 % ± 0.07 %
M+3	0.13 % ± 0.03 %
M+4	0.02 % ± 0.02 %
M+5	0.01 % ± 0.01 %
M+6	0.00 % ± 0.00 %

Supplemental Table S94 Relative fractions of isotopologues (mol%) of polar metabolites from experiments with with *F.* W12-1067 WT and Δmyo-inositol mutant grown in CDM medium supplemented with 9.13 mM myo-Inositol-C-d_6. M+x represents the mass M of the unlabelled metabolite plus x labelled D-atoms. Mean and SD from one experiment are shown.

9.13 mM myo-Inositol-C-d_6 *F.* W12-1067 WT					
	Lactate	Glycolic acid	Alanine	Succinic acid	Fumaric acid
M+1	2.49 % ± 0.05 %	1.00 % ± 0.15 %	2.56 % ± 0.07 %	1.98 % ± 0.19 %	1.19 % ± 0.06 %
M+2	4.82 % ± 0.02 %	1.20 % ± 0.19 %	0.62 % ± 0.05 %	2.59 % ± 0.04 %	0.03 % ± 0.03 %

M+3	0.14 % ± 0.01 %		0.02 % ± 0.01 %	0.05 % ± 0.03 %	
M+4	0.00 % ± 0.00 %		0.00 % ± 0.00 %	0.00 % ± 0.00 %	
M+5			0.00 % ± 0.00 %		

9.13 mM myo-Inositol-C-d_6 *F.* W12-1067 WT					
	Phenylalanine	Malic acid	Glutamate	Palmitic acid	Stearic acid acid
M+1	28.19 % ± 0.23 %	1.66 % ± 0.61 %	2.56 % ± 1.19 %	0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+2	10.38 % ± 0.17 %	0.00 % ± 0.00 %	0.69 % ± 0.75 %	2.31 % ± 0.69 %	3.11 % ± 1.14 %
M+3	3.04 % ± 0.08 %	0.00 % ± 0.00 %	0.20 % ± 0.29 %	0.13 % ± 0.22 %	0.00 % ± 0.00 %
M+4	0.42 % ± 0.06 %		0.44 % ± 0.11 %	0.07 % ± 0.04 %	0.21 % ± 0.06 %
M+5	0.50 % ± 0.06 %		0.01 % ± 0.01 %	0.01 % ± 0.02 %	0.02 % ± 0.01 %
M+6	0.33 % ± 0.03 %		0.00 % ± 0.00 %	0.04 % ± 0.01 %	0.07 % ± 0.04 %
M+7	0.00 % ± 0.00 %			0.01 % ± 0.01 %	0.01 % ± 0.01 %
M+8	1.14 % ± 0.06 %			0.02 % ± 0.01 %	0.02 % ± 0.02 %
M+9	0.07 % 0.01 %			0.00 % ± 0.01 %	0.00 % ± 0.01 %
M+10				0.02 % ± 0.01 %	0.00 % ± 0.00 %
M+11				0.05 % ± 0.04 %	0.00 % ± 0.00 %
M+12				0.02 % ± 0.02 %	0.45 % ± 0.12 %
M+13				0.01 % ± 0.02 %	0.06 % ± 0.03 %
M+14				0.00 % ± 0.00 %	0.03 % ± 0.04 %
M+1				0.03 ± 0.02	0.02 ± 0.03

5				% %	% %
M+16				0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+17				0.00 % ± 0.00 %	1.19 % ± 0.31 %
M+18				0.00 % ± 0.00 %	1.03 % ± 0.14 %
M+19				0.00 % ± 0.01 %	0.10 % ± 0.16 %
M+20				0.01 % ± 0.03 %	0.04 % ± 0.06 %
M+21				0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+22				0.10 % ± 0.10 %	0.01 % ± 0.01 %
M+23				0.02 % ± 0.00 %	0.00 % ± 0.00 %
M+24				0.79 % ± 0.05 %	0.00 % ± 0.00 %
M+25				0.26 % ± 0.02 %	0.00 % ± 0.00 %
M+26				0.11 % ± 0.01 %	0.02 % ± 0.03 %
M+27				0.04 % ± 0.02 %	0.00 % ± 0.00 %
M+28				0.11 % ± 0.04 %	0.02 % ± 0.02 %
M+29				0.00 % ± 0.00 %	0.01 % ± 0.01 %
M+30				0.00 % ± 0.00 %	0.00 % ± 0.00 %
M+31					0.00 % ± 0.00 %
M+32					0.00 % ± 0.00 %
M+33					0.01 % ± 0.02 %
M+34					0.02 % ± 0.03 %

M+35					0.00 % ± 0.00 %

9.13 mM myo-Inositol-C-d_6 *F.* W12-1067 Δmyo-inositol mutant				
	Lactate	Glycolic acid	Glutamate	Palmitic acid
M+1	0.29 % ± 0.06 %	0.22 % ± 1.48 %	0.61 % ± 0.95 %	0.00 % ± 0.00 %
M+2	1.13 % ± 0.01 %	0.65 % ± 0.69 %	0.37 % ± 0.63 %	0.27 % ± 0.11 %
M+3	0.11 % ± 0.01 %		0.19 % ± 0.26 %	0.11 % ± 0.01 %
M+4	0.00 % ± 0.01 %		0.45 % ± 0.10 %	0.06 % ± 0.01 %
M+5			0.01 % ± 0.02 %	0.00 % ± 0.01 %
M+6			0.00 % ± 0.00 %	0.03 % ± 0.01 %
M+7				0.01 % ± 0.00 %
M+8				0.02 % ± 0.01 %
M+9				0.01 % ± 0.00 %
M+10				0.02 % ± 0.01 %
M+11				0.03 % ± 0.02 %
M+12				0.01 % ± 0.01 %
M+13				0.02 % ± 0.01 %
M+14				0.00 % ± 0.00 %
M+15				0.00 % ± 0.00 %
M+16				0.02 % ± 0.01 %
M+17				0.00 % ± 0.00 %
M+18				0.00 % ± 0.00 %
M+19				0.00 % ± 0.00 %
M+20				0.00 % ± 0.00 %
M+21				0.00 % ± 0.00 %
M+22				0.05 % ± 0.02 %
M+23				0.01 % ± 0.01 %

M+24				0.43 % ± 0.05 %
M+25				0.10 % ± 0.01 %
M+26				0.02 % ± 0.02 %
M+27				0.01 % ± 0.00 %
M+28				0.04 % ± 0.02 %
M+29				0.01 % ± 0.01 %
M+30				0.00 % ± 0.00 %
M+31				0.00 % ± 0.01 %

Supplemental Table S95 Relative fractions of isotopologues (mol%) of sugarsfrom experiments with with *F.* W12-1067 WT and Δmyo-inositol mutant grown in CDM medium supplemented with 9.13 mM myo-Inositol-C-d_6. M+x represents the mass M of the unlabelled metabolite plus x labelled D-atoms. Mean and SD from one experiment are shown.

9.13 mM myo-Inositol-C-d_6 *F.* W12-1067 WT		
	Glucose in glycogen	
M+1	6.11 % ±	0.12 %
M+2	7.37 % ±	0.38 %
M+3	0.50 % ±	0.39 %
M+4	0.12 % ±	0.16 %
M+5	0.07 % ±	0.06 %
M+6	0.75 % ±	0.06 %

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Eidesstattliche Versicherung

Die experimentellen Arbeiten zur vorliegenden Dissertation wurden von mir, Fan Chen, selbständig im Zeitraum von Juli 2014 bis Juni 2018 an der Fakultät für Chemie, Lehrstuhl für Biochemie der Technischen Universität München durchgeführt. Die vorliegende Dissertation mit dem Titel „ Carbon fluxes and differential glucose usages in *Francisella* strains, *Bacillus subtilis* and thermogenic brown adipocytes" habe ich selbständig verfasst und mich außer der angegebenen keiner weiteren Hilfsmittel bedient.

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Ort, Datum Unterschrift