High Expression of Thyroid Hormone Receptors and Mitochondrial Glycerol-3-phosphate Dehydrogenase in the Liver Is Linked to Enhanced Fatty Acid Oxidation in Lou/C, a Rat Strain Resistant to Obesity*

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Besides its well recognized role in lipid and carbohydrate metabolisms, glycerol is involved in the regulation of cellular energy homeostasis via glycerol-3-phosphate, a key metabolite in the translocation of reducing power across the mitochondrial inner membrane with mitochondrial glycerol-3-phosphate dehydrogenase. Here, we report a high rate of gluconeogenesis from glycerol and fatty acid oxidation in hepatocytes from Lou/C, a peculiar rat strain derived from Wistar, which is resistant to age- and diet-related obesity. This feature, associated to Lou/C, a peculiar rat strain derived from Wistar, which is resistant to age- and diet-related obesity. This feature, associated to age- and diet-related obesity. This feature, associated to the well reported age-related obesity of their Wistar counterparts (5). Moreover, these animals also maintain their body mass in response to a high fat diet (6). Such features are only partly related to a spontaneous lower caloric intake (7, 8) because Lou/C rats have lower fat deposits but a higher percentage of carcass proteins and muscle mass as compared with pair-fed Wistar rats (4), even when subjected to a high fat diet (6). This peculiar metabolic phenotype has recently been investigated (9), showing lower blood glucose together with liver and muscle glycogen content, whereas insulin sensitivity was higher. These animals also exhibit a high basal activity, associated with a high capacity for long term exercise (9). Interestingly, when submitted to 60 min of exercise, these rats maintain blood glucose with no depletion of liver or skeletal muscle glycogen, in marked contrast to Wistar rats (9) and in accordance with a reported preference for fat oxidation as compared with carbohydrate (10). Preliminary experiments investigating liver gluconeogenesis revealed that hepatocytes from 24-h fasted Lou/C exhibited lower gluconeogenesis rates from most precursors (lactate-pyruvate, fructose, dihydroxyacetone, alanine), whereas glycerol was a remarkable exception with a higher rate of glucose production as compared with Wistar rats (9).

Glycerol is an important gluconeogenic substrate in liver and kidney because of high glycerol kinase activity (EC 2.7.1.30). However, muscle, brain, and other tissues also contain glycerol kinase (11, 12). Glycerol also has important implications via 1-glycerol-3-phosphate (G3P), which plays a key role in both lipid metabolism and energy homeostasis (11, 13). This latter effect is related to the transport of reducing equivalents across the inner mitochondrial membrane via the FAD-dependent mitochondrial glycerol-3-phosphate dehydrogenase (mGPDH, EC 1.1.99.5), and thanks to the presence of cytoplasmic NAD-dependent glycerol-3-phosphate dehydrogenase (cGPDH, EC 1.1.1.8), recycling occurs between G3P and dihydroxyacetone

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3 The abbreviations used are: G3P, glycerol-3-phosphate; cGPDH, cytosolic glycerol-3-phosphate dehydrogenase; mGPDH, mitochondrial glycerol-3-phosphate dehydrogenase; DHAP, dihydroxyacetone phosphate; DNP, 2,4-dinitrophenol; EF2, elongation factor 2; MCT-8, monocarboxylate transporter 8; TH, thyroid hormone; TRβ1, thyroid hormone receptor α1; TRβ1, thyroid hormone receptor β1; TSH, thyroid-stimulating hormone; HPLC, high pressure liquid chromatography; L/P ratio, lactate-to-pyruvate ratio.
phosphate (DHAP). This leads to a net transfer of reducing equivalents from cytosolic NADH to mitochondrial matrix (14) by reducing the quinone pool directly from mGPdH-linked FADH₂ (15). In the malate-aspartate carrier system, NADH is the final electron donor to the respiratory chain, whereas in the DHAP-G3P shuttle, NADH is converted to FADH₂, resulting in a substantial disparity between the two pathways regarding oxidative phosphorylation stoichiometry: three coupling sites with NADH versus two coupling sites with FADH₂. Hence, due to its role in the regulation of the respective flux through these two shuttles, G3P metabolism and its modulation by mGPdH activity play an important role in cellular energetic homeostasis. Indeed, knock-out mice for both mitochondrial and cytosolic GPdH died a few days after birth (16). mGPdH is present in most tissues of various animal species, but its activity varies from very high (brown adipose tissue and muscle) to very low (the heart and liver) (17). Thyroid hormones are potent activators of mGPdH transcription, especially in the liver, kidney, heart, skeletal muscle, diaphragm, and adipose tissue, but not in the brain, lungs, spleen, stomach, or small intestine (11, 17). Furthermore, steroid hormones (18), a low protein/high sucrose diet (19), long fasting, and acute cold exposure (20) also increase liver mGPdH. In addition to transcriptional regulation, calcium increases liver mGPdH activity by increasing enzyme affinity toward G3P (21).

In this study, we have investigated glycerol metabolism in freshly isolated liver cells from Lou/C and Wistar rats. A higher rate of glycerol metabolism was observed in Lou/C in comparison with Wistar. The main steps involved in this effect were glycerol phosphorylation and G3P oxidation, depending on the presence or not of fatty acids. We failed to find any significant change in glycerol kinase and cGPdH activities or mRNAs or in aquaglyceroporin 9 mRNA. By contrast, mGPdH activity, protein content, and transcripts were significantly higher in Lou/C. Moreover, we found significantly higher amounts of liver thyroid hormone receptor TRα1 protein and mRNAs, whereas circulating thyroid hormones were slightly lower in Lou/C. Higher octanoate metabolism and oxidative capacity are also reported. We proposed that the higher liver mGPdH activity represents an important feature of the metabolic phenotype of Lou/C.
strain, which may explain, at least in part, their resistance to obesity. The well known relationship between thyroid hormone and mGPdH transcription, associated with the present finding of higher liver thyroid hormone receptor mRNA in Lou/C, led us to propose it as a possible mechanism.

EXPERIMENTAL PROCEDURES

Isolation of Hepatocytes—Hepatocytes were prepared from male Wistar (250–350 g) and male Lou/C rats (220–280 g) aged 14 weeks according to the method of Berry and Friend (22) and modified by Groen et al. (23). 1 mg of dry hepatocytes equals \(\frac{1}{1100} \times 465,000\) cells.

Perifusion of Hepatocytes—Isolated hepatocytes (200–220 mg of dry cells) were perifused according to the method of Van Der Meer and co-workers (44) and modified by Groen (23) with increasing amounts of glycerol (from 0.15 to 9.6 mM), in the presence or not of 0.4 mM octanoate. At each steady state, perifusate and cell samples were collected. Glucose, lactate, pyruvate, 3-hydroxybutyrate, and acetoacetate were determined spectrophotometrically (24). Metabolite flux results were expressed in \(\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g dry cell}^{-1}\). Cell samples were collected from the perifusion chamber to separate the intracellular from the extracellular space by centrifugation through a layer of silicon oil (Rhodorsil 640 V 100) into HClO₄/EDTA (10% mass/volume, 25 mM) (25), and intracellular metabolites (fructose 6-phosphate, DHAP, and G3P) were determined (24). Mitochondrial and cytosolic compartments were separated by digitonin fractionation (26) for subsequent determination of adenine nucleotide content with the HPLC method.

Determination of Cellular Oxygen Consumption Rate—Hepatocytes (7.5 mg of dry cells/mL) were incubated in a shaking bath at 37 °C in closed vials containing 3.2 ml of Krebs Ringer bicarbonate calcium buffer without any substrates or after the addition of 20 mM glycerol, or 4 mM octanoate. Oxygen consumption rate (\(\text{JO}_2\)) was determined polarographically at 37 °C with a Clark electrode. \(\text{JO}_2\) was measured before and after the addition of oligomycin (6 \(\mu\text{g}\cdot\text{mL}^{-1}\)), 2,4 DNP (50 \(\mu\text{M}\)) and myxothiazol (3.8 \(\mu\text{M}\)).

Determination of Enzymatic Activities—Glycerol Kinase and cytosolic GPdH activity were measured spectrophotometrically in the supernatant of sonicated isolated hepatocytes (24). Mitochondrial GPdH activity was measured on the supernatant of isolated mitochondria after three cycles of freezing and thawing. Mitochondria were extracted according to the method of Klingenberg and Slenczka (27) in a sucrose medium (250 mM sucrose, 1 mM EGTA, 20 mM Tris, pH 7.2). 40 \(\mu\text{g}\) of mitochondria were incubated in a KH₂PO₄/K₂HPO₄ buffer (50 mM, pH 7.5) containing 9.3 \(\mu\text{M}\) antimycin A, 5 \(\mu\text{M}\) rotenone and decylubiquinon (50 \(\mu\text{M}\)). The reduction of dichloroindophenol (50 \(\mu\text{M}\)) by mGPDH was measured.
ured spectrophotometrically at 600 nm at 37 °C and is expressed as \( \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{mg of proteins}^{-1} \).

**Western Blot Analysis**—Samples of isolated hepatocytes were lysed, and proteins were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Expression of mGPdH and eukaryotic elongation factor 2 (EF2) were monitored by SDS-PAGE immunoblots using mGPdH (gift from Dr. J. Weitzel), EF2 (Santa Cruz Biotechnology), and TR-specific antibodies linked to horseradish peroxidase. Blots were developed using chemiluminescence (Roche Applied Science).

**RNA Purification and Reverse Transcription-coupled PCR**—RNA was extracted from isolated hepatocytes with the TRIzol RNA isolation reagent (Roche Diagnostics). Total RNA (1 \( \mu \text{g} \)) was reverse-transcribed, and quantitative real-time PCR was then performed with SYBR Green Core kit on a MyiQ thermal cycler (Bio-Rad). mRNA contents were normalized for actin mRNA and expressed relative to that of Wistar using the 2\(^{-\Delta \Delta C_{\text{T}}^{*}} \) method (28). MCT-8, THRα1, and THRβ1 were normalized to RSP12 as an internal standard.

**Confocal Microscopy**—Images of isolated hepatocytes were acquired with a Nikon TE 200 microscope equipped for epifluorescent illumination (xenon light source, 75 watts), associated with a 12-bit digital-cooled charged-coupled device camera (SPOT-RT, Diagnostic Instruments). The autofluorescence of NADH and FAD was obtained after the incubation of isolated hepatocytes without any substrates, with glycerol (10 mM), octanoate (2 mM), or DNP (75 \( \mu \text{M} \)) for 5 min.

**Measurement of Blood Parameters**—TSH, thyroxine (T4), and triiodothyronine (T3) were measured by radioimmunoassay with rat standard (Amersham Biosciences, radioimmunoassay FT4-immunotech, radioimmunoassay FT3-immunotech for TSH, T4, and T3, respectively).

**Statistics**—The results are expressed as mean ± S.E. Statistically significant differences were assessed by analysis of vari-

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**FIGURE 3. Gluconeogenesis from glycerol in the presence of octanoate is higher in hepatocytes from Lou/C.** Hepatocytes (220 mg of dry cells) isolated from 24-h-starved Wistar (closed symbols) and Lou/C rats (open symbols) were perifused with increasing concentrations of glycerol and 0.4 mM octanoate. The rates of gluconeogenesis (**A**, \( J_{\text{Glucose}} \)) and ketogenesis (**B**, \( J_{\text{acetoacetate}} \) and \( J_{\text{1-hydroxybutyrate}} \)) were calculated from the glucose and acetoacetate + 1-hydroxybutyrate concentration in the perifusate, respectively. The mitochondrial and cytosolic redox potentials were calculated from the ratio of the 1-hydroxybutyrate-to-acetoacetate (C, \( \beta \)-hydroxybutyrate/acetoacetate) and lactate-to-pyruvate (D) and the ratios, respectively. Cytosolic ATP-to-ADP (E) and mitochondrial AMP (F) were measured by HPLC. At each steady state (20 min), samples of cell suspension were removed from the perfusion chamber. They were then centrifuged through an oil layer into HClO. Intracellular DHAP (G) and G3P (H) were measured enzymatically in the neutralized acid-soluble fraction and plotted as \( J_{\text{Glucose}} \). The results are expressed as means ± S.E., \( n = 5 \). *, \( p < 0.05 \) between Wistar and Lou/C.

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**TABLE 1.** Gluconeogenic and ketogenic rates of isolated hepatocytes from Wistar and Lou/C rats (220 mg of dry cells) perifused with increasing concentrations of glycerol and 0.4 mM octanoate.

| Concentration (mmol/L) | Wistar | Lou/C |
|------------------------|--------|-------|
| Glycerol               |        |       |
| 0                      |        |       |
| 10                     |        |       |
| 20                     |        |       |
| 40                     |        |       |
| 60                     |        |       |

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**TABLE 2.** Cytosolic and mitochondrial redox potenti**
steady states; each rate of substrate infusion generates an equal flux through every step, which is the rate of glucose formation \( \nu_{\text{glucose}} \). Therefore, by determining \( I_{\text{glucose}} \) at each steady state, we can infer this value to each step of the pathway. From the data presented in Fig. 1, \( G \) and \( H \), it appears that the steps located downstream of DHAP were not the location of any difference between the two strains because we found a single relationship between substrate concentration and related flux in both groups. Considering G3P oxidation (Fig. 1A), the unique relationship between G3P concentration and its metabolism exhibited a biphasic shape; the rate of G3P oxidation declined at G3P concentrations higher than 15 \( \mu \text{mol} \cdot \text{g}^{-1} \) of dry cells \(^{-1} \). This is probably the consequence of glucose 6-phosphatase (EC 3.1.3.3) inhibition by G3P (33).

Because gluconeogenesis from glycerol requires a stoichiometric utilization of ATP and \( \text{NAD}^+ \), higher \( I_{\text{glucose}} \) in Lou/C must be associated with a higher mitochondrial oxidative phosphorylation rate. Indeed, cellular oxygen consumption rate was significantly higher in hepatocytes of Lou/C as compared with Wistar rats with endogenous substrate (Fig. 2A) and with glycerol (Fig. 2B). The difference between the strains was found at basal (physiological) state, upon oligomycin addition (an inhibitor of ATP synthesis) and in respiration uncoupled by 2,4-dinitrophenol. However, when mitochondrial respiration was fully inhibited by myxothiazol, a complex 3 inhibitor, no difference was observed, indicating that the mitochondrial respiratory chain is the location of the increased oxygen consumption in Lou/C. Cytosolic ATP (Fig. 1F) and ATP-to-ADP ratio (ATP/ADP ratio) (Fig. 1F) were significantly higher in Lou/C. Taken together, these results indicate that a higher gluconeogenesis rate from glycerol in Lou/C hepatocytes is accompanied by a higher respiration rate, L/P ratio, and cytosolic ATP/ADP ratio.

Glycerol and Octanoate Metabolism in Lou/C, the Effect of High Reducing Power—Fatty acid metabolism dramatically decreases gluconeogenesis from glycerol because of the reducing effect (30, 31). Therefore, the combination of glycerol and fatty acid, a physiologically relevant situation, represents a suitable metabolic condition to further characterize the metabolic relationship between cytosolic redox state and hepatic glycerol metabolism in Lou/C. As expected, octanoate addition resulted
in a dramatic inhibition of gluconeogenesis from glycerol in both strains (compare Fig. 3A with Fig. 1A). Indeed, the lowering effect of octanoate metabolism on $J_{\text{Glucose}}$ is significantly less in Lou/C ($p < 0.005$) as compared with Wistar. Octanoate metabolism was higher in Lou/C, as indicated by higher rates of ketogenesis (Fig. 3B) and oxygen consumption (Fig. 2C). Moreover, a unique relationship was found between DHAP and $J_{\text{Glycerol}}$ regardless of the strain, whereas two different relationships were evidenced between G3P and $J_{\text{Glycerol}}$ (Fig. 3G), contrasting with the finding obtained with glycerol alone (Fig. 1J). Because these two relationships characterize glycerol pathways up- and downstream of DHAP, respectively, this result pointed to the step of G3P oxidation as the main target for the difference between Lou/C and Wistar in these conditions of glycerol and fatty acids. Indeed, an effect located upstream of the G3P dehydrogenase step leads to a single relationship between $J_{\text{Glycerol}}$ and G3P, whereas an effect located downstream of DHAP leads to a double relationship between $J_{\text{Glycerol}}$ and DHAP. As evidenced in Fig. 3D and the inset, octanoate metabolism markedly reduced the cytosolic compartment, as indicated by the rise in lactate-to-pyruvate ratio. This effect is significantly more pronounced in the control Wistar in comparison with Lou/C ($p < 0.05$ between the two strains). The production in Lou/C appeared to be related to a redox effect located at the G3P oxidation step, contrasting with the finding above in which glycerol was the unique exogenous substrate.

**In Situ Hepatocyte Redox State Assessment by Nucleotide Autofluorescence**—Assessment of cytosolic and mitochondrial redox state is not accurately evaluated from direct determination of reduced and oxidized nucleotide contents because the ratio between free and bound metabolites is not accessible; therefore, an indirect method based on the measurement of lactate-to-pyruvate or $\beta$-hydroxybutyrate-to-acetoacetate ratios was proposed (32). However, by taking advantage of the specific autofluorescence of NADH and FAD (see “Experimental Procedures” and Fig. 4), an assessment of the cellular redox state could also be achieved in freshly isolated liver cells by confocal microscopy. NADH fluorescence (blue) decreases while oxidized to NAD, whereas flavin fluorescence (green) increases when oxidized (FAD); therefore, a decrease in the blue-to-green ratio reflects a more oxidized state. The decrease in the blue/green fluorescence ratio following uncoupling by DNP (shown in Fig. 4) indicated the physiological relevance of this assessment of the global cellular redox state, which included nicotinamide and flavin cofactors. The fluorescence ratio was significantly lower in Lou/C in comparison with Wistar, reflect-
Molecular Targets Involved in the Modification of Glycerol Metabolism in Lou/C Rats—As commonly recognized, and accordingly to the data presented above, the main controlling steps of gluconeogenesis from glycerol are glycerol phosphorylation and G3P oxidation; however, glycerol transport across the plasma membrane might also play a role. The membrane-bound aquaglyceroporins are recognized as glycerol carriers, and aquaglyceroporin 9 has been proposed as being specific to the liver (36). Similar transcript levels were found in both strains (Fig. 5D), indicating that glycerol transport across the cellular membrane was probably similar in both strains. Potentially, G3P oxidation depends on both cGPDH and mGPDH, with activity of the latter being very low in the liver (29). Glycerol kinase (9.8 ± 0.5 versus 9.2 ± 0.2 μmol/min·1·g of dry cells for Wistar and Lou/C, respectively, n = 5) and cGPDH (70.25 ± 4.78 versus 65.42 ± 9.84 μmol/min·1·mg of proteins for Wistar and Lou/C, respectively, n = 5) activities and transcripts (Fig. 5A) were similar in both strains. By contrast, mGPDH activity (+70%, p < 0.05, Fig. 5B), protein, and transcript contents (+100%, p < 0.01) were markedly higher in Lou/C. The transcription of peroxisome proliferator-activated receptor-α (PPARα), a factor involved in the expression of several genes involved in glycerol metabolism including mGPDH (37), was no different between the two strains, excluding its implication in the higher expression of mGPDH in Lou/C (Fig. 5D).

THs are known as potent activators of mGPDH transcription in the liver. However, plasma concentrations of T3 and T4 were slightly but significantly lower in Lou/C rats, whereas TSH was higher (Fig. 5E), a finding already reported in this strain (38). Interestingly, the assessment of TH receptors TRα1 and TRβ1 in livers from Wistar and Lou/C revealed significantly higher TRα1 protein (Fig. 5G) and TRα1 and TRβ1 mRNA levels in Lou/C (Fig. 5F). Several other targets dependent on TR receptor activation (Fig. 5F, Spot 14 (S14), cytochrome oxidase (COX), F1-ATPase, mitochondrial transcription factor A (mTFA), and deodain 1 (Dio1)) were also modified, indicating a general effect (Fig. 5F). In addition, we have investigated in vivo the effect of an exogenous load of T3 on mGPDH transcription level in the two strains Wistar and Lou/C. It was found that basal as well as T3-stimulated levels of mGPDH transcripts were significantly higher in Lou/C, whereas plasma T3 levels were similar (data not shown).

**DISCUSSION**

Higher TH receptors associated with a higher mGPDH activity in the liver from the Lou/C strain represent the main findings of the present work. This feature appears to have a substantial effect on hepatic fatty acid oxidation and glycerol metabolism. This may, at least to some extent, explain the key physiological characteristics of this peculiar strain. Indeed, modulating the pathway of electron supply to the respiratory chain has considerable implications on whole-body energy homeostasis. In comparison with Wistar, Lou/C strain is characterized by a spontaneous lower body mass and food intake. However,
unlike pair-fed animals, muscle mass is proportionally higher, whereas fat mass is lower, indicating disturbances other than food intake. Impressive resistance to diet- and age-related obesity with hyperactivity associated with an exceptional ability for long term physical exercise represent the most characteristic features of this animal (6, 39). The reported increase in mGPdH activity is most likely a transcriptional effect because both transcripts and protein were higher. A local (hepatic) increase in TH effects, due to higher levels of TRα1 and TRγ1, probably explains such an effect occurring despite low circulating T3 and T4 levels.

The higher gluconeogenic rate from glycerol in Lou/C is due to an effect located upstream of G3P oxidation and associated with a higher cytosolic ATP/ADP ratio. The absence of any difference in aquaglyceroporin 9 transcripts does not point to an effect on glycerol transport. The flux of glycerol phosphorylation is higher in Lou/C despite the fact that it has a similar glycerol kinase content, activity, and transcription. This is probably explained by the higher cytosolic ATP/ADP ratio (11, 15). The association of a higher mGPdH activity with a high ATP/ADP ratio in Lou/C is interesting because the opposite was expected in light of the decreased oxidative phosphorylation stoichiometry resulting from a higher G3P shuttle. ATP levels and the ATP/ADP ratio depend on both the rate of ATP synthesis and the utilization of ATP rather than on oxidative phosphorylation efficiency. In fact, we have shown that mitochondrial adaptation to various cellular energy demands is based on a permanent compromise between rate and efficiency; the highest rate is achieved at the lowest efficiency and vice versa (40). On one hand, bypassing complex 1 by mGPdH lowers ATP-to-oxygen stoichiometry (ATP/O) as complex 1, i.e. the first coupling site of the respiratory chain, is excluded from the pathway. However, on the other hand, due to the absence of any flux controlling effect of complex 1, a higher respiratory rate is achieved, explaining the higher rate of ATP synthesis despite lower efficiency (40, 41). Octanoate addition to glycerol further reduces cytosolic compartment (compare Figs. 1D and 3D), whereas glucose production and ATP/ADP ratio decrease (compare Figs. 1A, A and F, and 3A and E) in both strains. However, the lowering effect of octanoate on gluconeogenesis and its reducing effect are less pronounced in Lou/C as compared with Wistar. This feature probably results from a higher rate of NADH oxidation via the FAD-dependent G3P shuttle activation in Lou/C. Thus, as proposed above for glycerol metabolism, in the case of glycerol and octanoate, higher mGPdH activity in Lou/C might also explain an increased fatty acid oxidation in this strain.

The present work emphasizes the role of a reducing-power translocation pathway across the mitochondrial inner membrane in the regulation of mitochondrial oxidative phosphorylation and fatty acid oxidation. Two pathways carry reducing power into the mitochondrial matrix: the malate-aspartate and the G3P-DHAP shuttles (Fig. 6). Although the former is thermodynamically controlled by mitochondrial membrane potential because of the ΔPm-dependent glutamate transport, the latter is kinetically controlled in the liver by a very low mGPdH activity (17). When electrons are carried by the malate-aspartate shuttle, high oxidative phosphorylation stoichiometry (three coupling sites) is achieved at a limited rate of respiration and ATP synthesis because of the control exerted at complex 1. By contrast, G3P shuttle provides electrons from cytosolic NADH to the quinone pool, i.e. downstream of complex 1. This results in a lower stoichiometry (two coupling sites only) but at a higher rate of respiration and ATP synthesis. Because malate-aspartate shuttle flux is dependent on the mitochondrial membrane potential ΔPm, it is sensitive to any process affecting the protonmotive force, such as uncoupling. This is not the case for G3P shuttle flux because it does not depend on ΔPm, but mostly on mGPdH activity in the liver. Therefore, uncoupling would favor FAD-linked substrate oxidation (42, 43).

The high rate of fatty acid oxidation in Lou/C, which was related to the high mGPdH activity, may explain the resistance to obesity. This metabolic feature appears to be related to a “hyperthyroid status” limited to the liver, resulting from the high TH receptor transcription. The mechanism that leads to overexpression of TH receptors in this intriguing strain remains to be clarified, but this finding opens a new direction in the field of obesity and energy homeostasis.

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