



# Enzymes of glycerol and glyceraldehyde metabolism in mouse liver: effects of caloric restriction and age on activities

Kevork HAGOPIAN\*<sup>1</sup>, Jon J. RAMSEY\* and Richard WEINDRUCH†‡

\*Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA 95616, U.S.A., †Department of Medicine, University of Wisconsin Medical School, Madison, WI 53706, U.S.A., and ‡Geriatric Research, Educational and Clinical Center, William S. Middleton Memorial Veterans Hospital, 2500 Overlook Terrace, Madison, WI 53705, U.S.A.

## Synopsis

The influence of caloric restriction on hepatic glyceraldehyde- and glycerol-metabolizing enzyme activities of young and old mice were studied. Glycerol kinase and cytoplasmic glycerol-3-phosphate dehydrogenase activities were increased in both young and old CR (calorie-restricted) mice when compared with controls, whereas triokinase increased only in old CR mice. Aldehyde dehydrogenase and aldehyde reductase activities in both young and old CR mice were unchanged by caloric restriction. Mitochondrial glycerol-3-phosphate dehydrogenase showed a trend towards an increased activity in old CR mice, whereas a trend towards a decreased activity in alcohol dehydrogenase was observed in both young and old CR mice. Serum glycerol levels decreased in young and old CR mice. Therefore increases in glycerol kinase and glycerol-3-phosphate dehydrogenase were associated with a decrease in fasting blood glycerol levels in CR animals. A prominent role for triokinase in glyceraldehyde metabolism with CR was also observed. The results indicate that long-term caloric restriction induces sustained increases in the capacity for gluconeogenesis from glycerol.

**Key words:** aging, caloric restriction, energy source, glyceraldehyde metabolism, glycerol metabolism, liver

## INTRODUCTION

Glycerol and glyceraldehyde are two metabolites of intermediary metabolism, resulting from the breakdown of triacylglycerols and fructose respectively. These metabolites have important roles, acting as the links between several metabolic pathways, and undergo further metabolism by entering into these pathways. Glycerol is produced as a result of the breakdown of proteins, triacylglycerols and other glycerolipids, as well as dietary fats (reviewed in [1–4]). It is also formed from de-esterification of triacylglycerols in adipose tissues, skeletal muscle, liver and blood [5], with de-esterification in adipose tissue and blood accounting for the majority of circulating glycerol [6,7]. Glycerol-metabolizing enzymes are essentially tissue-specific, with GK (glycerol kinase), G3PDH (glycerol-3-phosphate dehydrogenase) and ADH (alcohol dehydrogenase) found predominantly in liver [1,7]. Glycerol is utilized as an important gluconeogenic substrate, mainly in the liver and kidneys. It is converted into G3P (glycerol 3-phosphate) by phosphorylation, which is the more physiologically important form of glycerol, and then

into DHAP (dihydroxyacetonephosphate), which is catalysed by NAD-dependent cG3PDH (cytosolic G3PDH), therefore facilitating its entry into the glycolytic pathway. Also, G3P is metabolized by mitochondrial FAD-dependent mG3PDH (mitochondrial G3PDH), part of the G3P shuttle, which is found on the outer surface of the inner membrane. cG3PDH and mG3PDH form the glycerol–phosphate shuttle, which plays an important role in tissues that oxidize glucose rapidly, such as brain [8], skeletal muscle [9] and the flight muscle of insects [10], regenerating NAD from NADH formed by glycolysis. This shuttle, however, does not play an important role in other mammalian systems, such as liver and kidneys, where the malate–aspartate shuttle is the predominant one [1,4,11]. Glycerol is also metabolized by ADH to D-glyceraldehyde, as well as undergoing re-esterification to form triacylglycerols. It has been reported that in humans, under normal conditions, the contribution of glycerol to gluconeogenesis is less than 5%, but increasing to more than 20% after 2–3 days of starvation and becoming the primary source for gluconeogenesis during prolonged fasting, as glycogen stores are depleted within 2 days [6,12]. Under conditions of starvation, glycerol provided almost 80% of new glucose in obese individuals, as opposed

**Abbreviations used:** ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; ALR, aldehyde reductase; CR, calorie-restricted; DHAP, dihydroxyacetonephosphate; F-1,6-BPase, fructose 1,6-bisphosphatase; F-2,6-BP, fructose 2,6-bisphosphate; GAP, glyceraldehyde 3-phosphate; GK, glycerol kinase; G3P, glycerol 3-phosphate; (c/m)G3PDH, (cytosolic/mitochondrial) glycerol-3-phosphate dehydrogenase; PFK-1, 6-phosphofructo-1-kinase; PPAR, peroxisome-proliferator-activated receptor; TK, triokinase.

<sup>1</sup>To whom correspondence should be addressed (khagopian@ucdavis.edu).



to 38% in lean individuals [6,13]. The increased utilization of glycerol for gluconeogenesis during starvation, due to lipolysis, was attributed to increased hepatic GK and G3PDH activities [14]. Although there is considerable information about glycerol metabolism during starvation, it is not known if its metabolism is altered with sustained caloric restriction.

Glyceraldehyde, on the other hand, is produced from the breakdown of F1P (fructose 1-phosphate), connecting fructose metabolism to that of glycerol and to glycolysis and gluconeogenesis. Therefore glyceraldehyde can be metabolized via three different routes [15]: first, it can be converted into GAP (glyceraldehyde 3-phosphate) by TK (triose kinase), and enters the glycolytic pathway. Second, it can be converted into D-glycerate by ALDH (aldehyde dehydrogenase) which, after further conversion by GlyK (glycerate kinase), also enters the glycolytic pathway. Third, it can be converted into glycerol by ADH. Of these three possible routes, the one catalysed by TK is the most dominant one [16,17]. Moreover, the conversion of glyceraldehyde into glycerol can also be achieved by the action of ALR (aldehyde reductase) [18] (which is also known as NADP-dependent ADH). Although the pathways for glyceraldehyde metabolism have been clearly defined, it is not known if energy intake plays a role in regulating the activity of these pathways.

Caloric restriction, without malnutrition, is the only intervention that has been shown consistently to delay pathophysiological changes and extend maximum life span in a variety of organisms [19,20]. This association between caloric-restriction-related increases in longevity and lowered age-related pathophysiological changes has been known for over 70 years [21]. The effects of caloric restriction appear to be dependent on an overall decrease in energy intake, rather than a decrease in any particular nutrient [22]. Therefore the changes in energy metabolism must clearly play a central role in the actions of caloric restriction and the ability of the animal to survive sustained caloric restriction. The adaptations in energy metabolism under sustained CR (calorie-restricted) conditions have not been well defined, and it is not known if changes in energy metabolism occur after dynamic weight loss has ceased. In previous studies [17,23–26], we have reported the influence of caloric restriction on other major metabolic pathways. The aim of the present study was to characterize and establish the effects of long-term caloric restriction and aging on the glycerol- and glyceraldehyde-metabolizing enzymes. The pathways for the metabolism of these two metabolites are interconnected with each other and with pathways that have been studied previously; therefore, it would be of interest to see if these two metabolites do contribute to the provision of alternative energy sources during caloric restriction.

## MATERIALS AND METHODS

### Materials

All laboratory chemicals and auxiliary enzymes were purchased from Sigma (St. Louis, MO, U.S.A.) or Roche (Indianapolis,

IN, U.S.A.). The protein assay kit was from Bio-Rad (Hercules, CA, U.S.A.).

### Animals

Male C57Bl/6J mice were purchased from Charles River Laboratories (Wilmington, MA, U.S.A.) at 1 month of age and housed individually at 23°C and with a 12 h light/12 h dark cycle. Animals were maintained in accordance with the local and federal guidelines governing animal experimentation. The mice were fed *ad libitum* a non-purified diet, PLI 5001 (Purina Laboratories, St. Louis, MO, U.S.A.) for 1 month, and at 2 months of age they were assigned either to the control or CR group and fed semi-purified diets as described previously [27]. At the time of killing, young animals were 3 months of age (1 month on either the control or CR diet) and old animals were 30 months of age (28 months on either the control or CR diet). All parameters were measured for six animals for each age and diet group. CR mice were fed a 30% restricted diet, and all feeding procedures have been described previously [25].

### Tissue harvesting and preparation

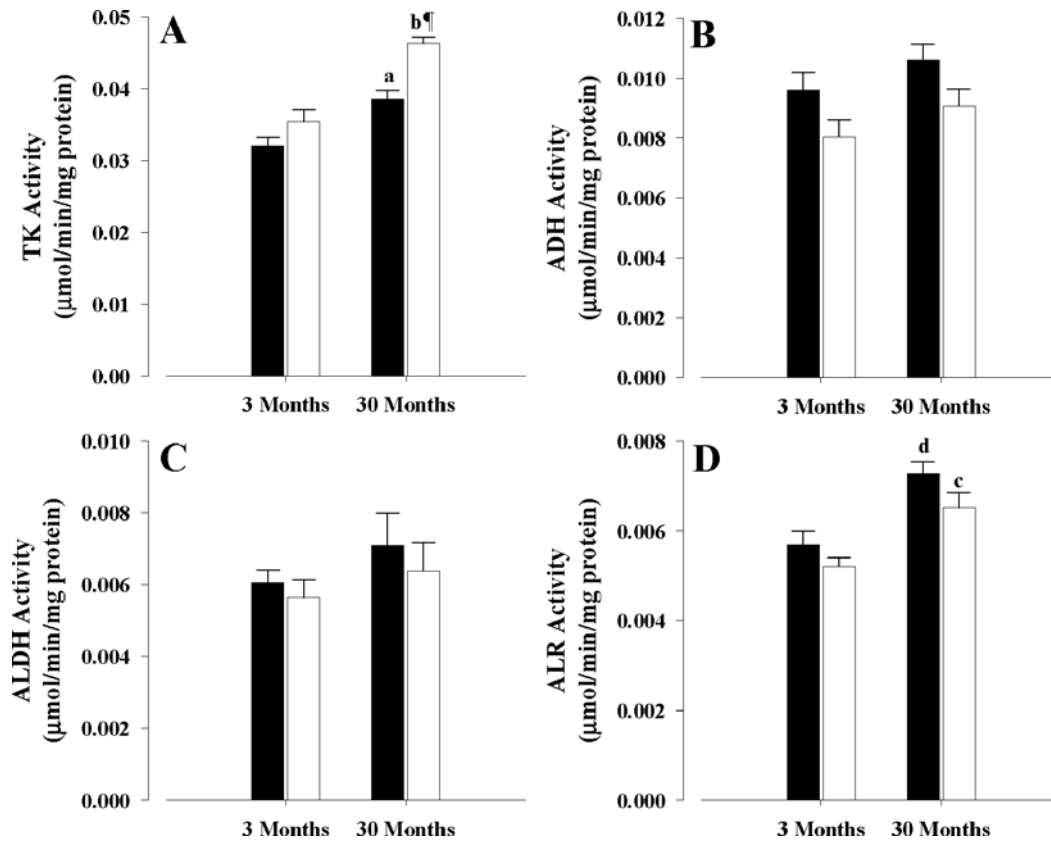
After an overnight fast, all mice were killed and their livers harvested between 9:00–10:00 h, as described previously [25]. Livers were rapidly freeze-clamped *in situ*, placed immediately in liquid nitrogen, powdered under liquid nitrogen in a mortar and pestle, and stored in liquid nitrogen for future use. Blood was collected immediately after decapitation, from the severed neck blood vessels, and the serum was prepared as described previously [28]. Body masses for old control and CR mice were  $31.13 \pm 1.34$  g and  $21.91 \pm 0.52$  g respectively, and  $18.68 \pm 0.48$  g and  $15.31 \pm 0.59$  g for young control and CR mice respectively. Liver masses for old control and CR mice were  $1.73 \pm 0.06$  g and  $1.19 \pm 0.03$  g respectively, and  $1.14 \pm 0.03$  g and  $0.91 \pm 0.04$  g for young control and CR respectively. The differences between control and CR body masses, in both young and old mice, were statistically significant, as were the liver masses ( $P < 0.001$ ).

### Measurement of enzyme activities

The powders, which had been stored in liquid nitrogen, were weighed and homogenized at a 1:10 ratio (w/v) and the supernatants were saved for assays. Glycerol- and glyceraldehyde-metabolizing enzymes that were assayed included: GK (EC 2.7.1.30) [29], cG3PDH (EC 1.1.1.8) [30], mG3PDH (EC 1.1.99.5) [31], NAD-dependent ADH (EC 1.1.1.1) [32], ALR (also known as NADP-dependent ADH, EC 1.1.1.2) [33], ALDH (EC 1.2.1.3) [34] and TK (EC 2.7.1.28) [35]. All assays were performed using a Lambda 25 UV/VIS spectrophotometer (PerkinElmer) and activities were expressed as  $\mu\text{mol}/\text{min}$  per mg of protein.

### Measurement of metabolites

For liver metabolites, the powders, which had been stored in liquid nitrogen, were weighed, homogenized in ice-cold perchloric acid (6%, w/v) and centrifuged, and the supernatants were removed and neutralized [23], and the levels of DHAP [23] and



**Figure 1** Activities of glyceraldehyde-metabolizing enzymes

Activities were measured as described in the text. (A) TK, (B) ADH, (C) ALDH and (D) ALR. All activities are expressed as the means  $\pm$  S.E.M for at least six independent experiments and expressed as  $\mu\text{mol}/\text{min}$  per mg of protein. Controls, filled bars; CR mice, open bars. Comparisons between CR and control mice:  $†P < 0.001$  old CR versus old control. Comparisons between age groups on similar diets: <sup>a</sup> $P < 0.003$  old control versus young control; <sup>b</sup> $P < 0.001$  old CR versus young CR; <sup>c</sup> $P < 0.01$  old CR versus young CR; <sup>d</sup> $P < 0.01$  old control versus young control.

G3P [36] were determined. Glycerol was determined in serum according to the method described by Wieland [37]. Liver metabolites were expressed as  $\mu\text{mol}/\text{g}$  of wet mass, and blood glycerol was expressed as  $\mu\text{mol}/\text{ml}$  of serum.

### Other methods

Protein concentrations were measured with a protein assay kit (BioRad), using BSA as the standard. Statistical comparisons were performed using Student's *t* test ( $P \leq 0.05$  was statistically significant).

## RESULTS

### Glyceraldehyde pathway

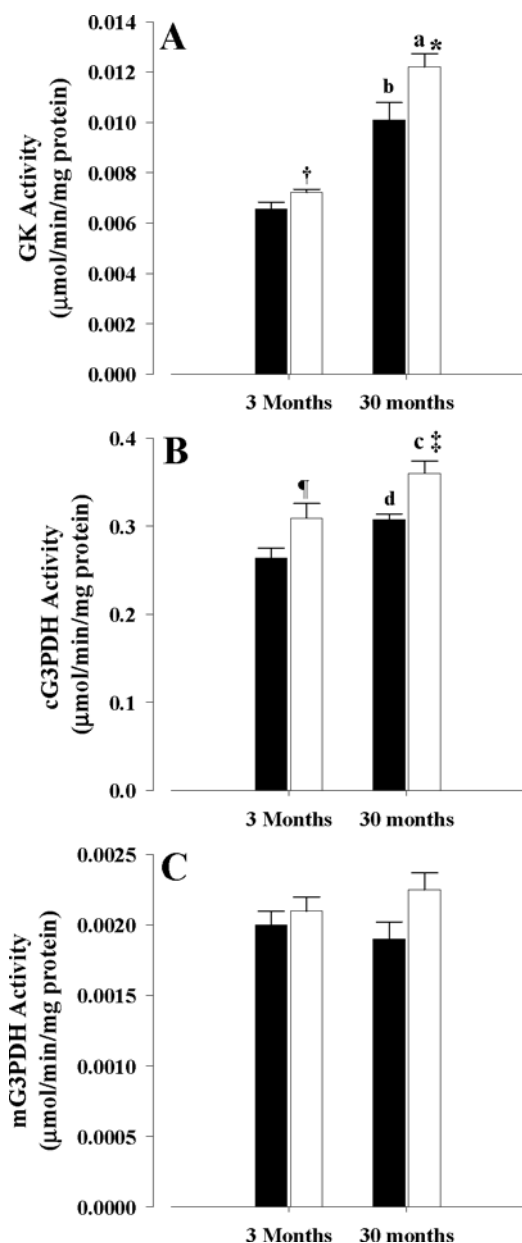
The activities of the glyceraldehyde pathway enzymes are shown in Figure 1. Old CR mice showed a 20% increased ( $P < 0.001$ ) TK activity (Figure 1A) and a trend towards a decrease ( $P = 0.08$ ) in ADH activity (Figure 1B) when compared with old controls.

In the case of ALDH, no significant differences in the activity between old CR and control mice were observed (Figure 1C), as was the case for ALR (Figure 1D). In young mice, on the other hand, no differences were observed between CR and control activities for TK (Figure 1A), ALDH (Figure 1C) and ALR (Figure 1D); however, ADH in young CR (Figure 1B) showed a trend ( $P = 0.085$ ) towards a lower activity when compared with young controls.

With regards to the effects of age on the activities, TK showed a significantly higher activity in old control (20%,  $P < 0.003$ ) and old CR (31%,  $P < 0.001$ ) mice when compared with corresponding young mice (Figure 1A), whereas ADH (Figure 1B) and ALDH (Figure 1C) showed no age-related changes in activity when young and old mice were compared. In the case of ALR (Figure 1D), old control and CR mice were 28% ( $P < 0.01$ ) and 25% ( $P < 0.01$ ) respectively higher in their activities than corresponding young mice.

### Glycerol pathway

For the glycerol pathway, old CR mice showed a 21% increase ( $P < 0.03$ ) in GK (Figure 2A) and 17% increase ( $P < 0.01$ ) in



**Figure 2 Activities of glycerol-metabolizing enzymes**

Activities were measured as described in the text. (A) GK, (B) cG3PDH and (C) mG3PDH. All activities are expressed as the means  $\pm$  S.E.M for at least six independent experiments and expressed as  $\mu\text{mol}/\text{min}$  per mg of protein. Controls, filled bars; CR, open bars. Comparisons between CR and control mice: \* $P < 0.03$  old CR versus old control; † $P < 0.05$  young CR versus young control; ‡ $P < 0.01$  old CR versus old control; ¶ $P < 0.05$  young CR versus young control. Comparisons between age groups on similar diets: <sup>a</sup> $P < 0.001$  old CR versus young CR; <sup>b</sup> $P < 0.001$  old control versus young control; <sup>c</sup> $P < 0.045$  old CR versus young CR; <sup>d</sup> $P < 0.01$  old control versus young control.

cG3PDH activities (Figure 2B) when compared with old controls, whereas a trend ( $P = 0.075$ ) toward an increase in mG3PDH was observed in old CR mice when compared with old controls (Figure 2C). In young CR mice, a similar pattern was also observed, with both GK (Figure 2A) and cG3PDH (Figure 2B) showing

10% ( $P < 0.05$ ) and 17% ( $P < 0.05$ ) increases in their activities respectively when compared with young controls, whereas mG3PDH (Figure 2C) was unchanged between young control and CR mice. The other two enzymes involved in glycerol metabolism, namely ADH and ALR, are the same ones involved in the glyceraldehyde metabolism and the results for both old and young CR were the same as those described above (Figures 1B and 1D).

For age-related changes in activities, GK activities (Figure 2A) were significantly higher ( $P < 0.001$ ) in both old control and CR mice when compared with corresponding young mice. This was also the case with cG3PDH (Figure 2B), where higher activities were observed for old CR ( $P < 0.045$ ) and old control ( $P < 0.01$ ) when compared with corresponding young mice. However, no age differences were observed for mG3PDH (Figure 2C) between young and old mice, whereas ADH and ALR activities were described above for the glyceraldehyde pathway (Figures 1B and 1D).

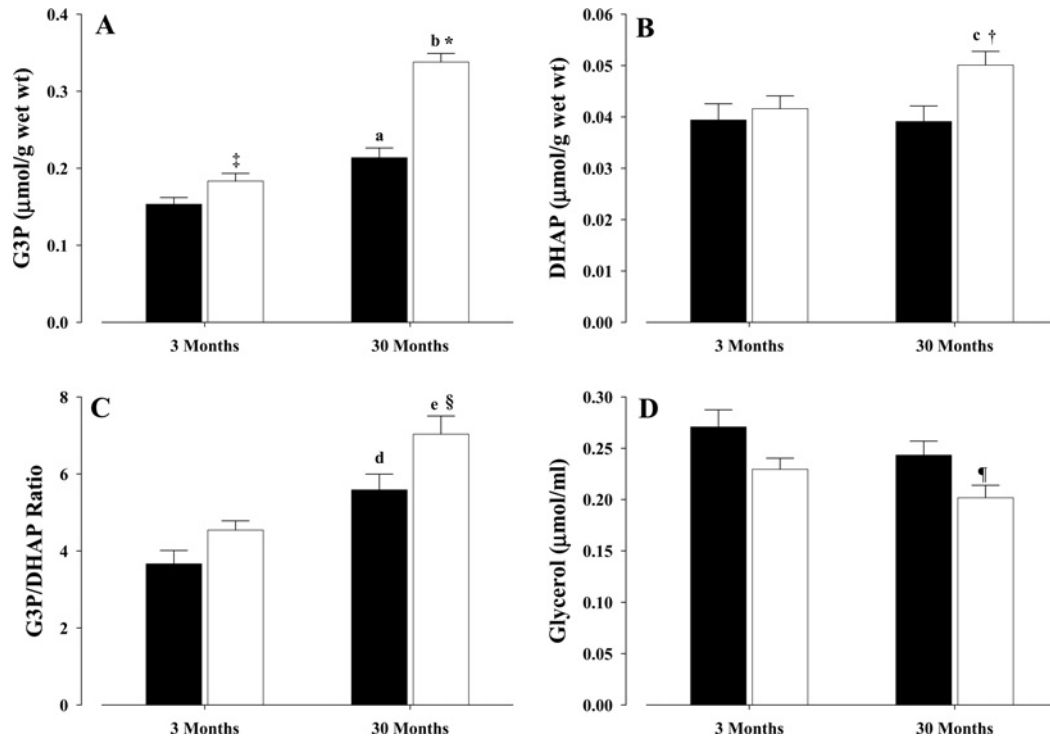
### Metabolite levels

In old CR mice, liver metabolites showed significantly increased levels of G3P ( $P < 0.001$ ) and DHAP ( $P < 0.02$ ), as well as an increased G3P/DHAP ratio ( $P < 0.03$ ), when compared with old controls (Figures 3A–3C respectively). Serum glycerol levels decreased significantly ( $P < 0.045$ ) in old CR mice when compared with old controls (Figure 3D). In young CR mice, G3P was increased significantly ( $P < 0.05$ ), although not as dramatically as in the old CR mice (Figure 3A), whereas no differences between young CR and control mice were observed for DHAP (Figure 3B). As for the G3P/DHAP ratio (Figure 3C), a trend ( $P = 0.065$ ) towards an increase in young CR mice was observed when compared with young controls. For serum glycerol levels (Figure 3D), a trend towards a decrease ( $P = 0.06$ ) was observed in young CR mice when compared with young controls.

The influence of age was also determined, with significantly higher levels of G3P in old control ( $P < 0.003$ ) and old CR ( $P < 0.0001$ ) mice than in the young (Figure 3A). In the case of DHAP, old CR mice showed significantly higher levels ( $P < 0.04$ ) than the young CR animals, whereas no differences were observed between old and young controls (Figure 3B). The G3P/DHAP ratio also increased significantly with age, with old control and old CR mice having higher ratios than the corresponding young mice ( $P < 0.004$  and  $P < 0.04$  respectively) (Figure 3C). Differences in serum glycerol levels were insignificant between young and old controls and between young and old CR mice (Figure 3D).

## DISCUSSION

Glyceraldehyde and glycerol are at the crossroads of several interconnecting pathways (Figure 4). To our knowledge, there are no previous reports concerning the effects of caloric restriction on the enzymes investigated in the present study, except for one



**Figure 3** Hepatic G3P, DHAP, G3P/DHAP ratio and serum glycerol levels in mice

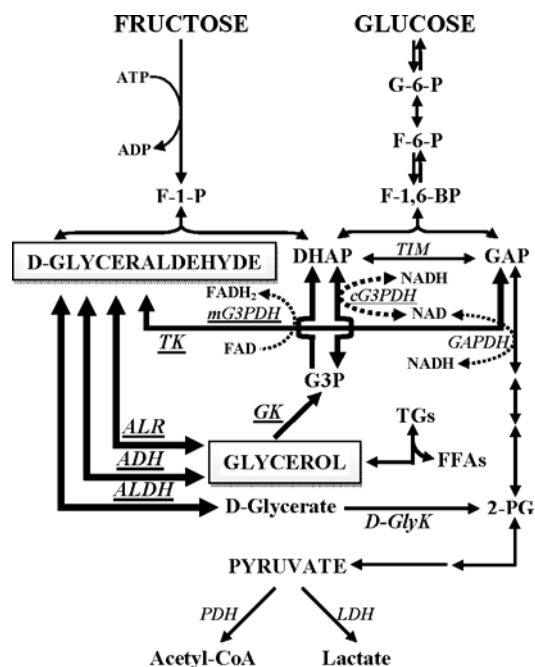
Hepatic metabolites and serum glycerol levels were determined as described in the text. (A) G3P (B) DHAP, (C) G3P/DHAP ratio and (D) serum glycerol. All values are expressed as the means  $\pm$  S.E.M for at least six independent experiments. Controls, filled bars; CR, open bars. Comparisons between CR and control mice: \* $P < 0.001$  old CR versus old control, † $P < 0.05$  young CR versus young control, ‡ $P < 0.02$  old CR versus old control, § $P < 0.03$  old CR versus old control, ¶ $P < 0.045$  old CR versus old control. Comparisons between age groups on similar diets: <sup>a</sup> $P < 0.003$  old control versus young control, <sup>b</sup> $P < 0.0001$  old CR versus young CR, <sup>c</sup> $P < 0.04$  old CR versus young CR, <sup>d</sup> $P < 0.004$  old control versus young control, <sup>e</sup> $P < 0.04$  old CR versus young CR.

previous study of TK under control and CR conditions [17]. The present work is the first to describe the activities of the enzymes involved in the metabolism of these two metabolites under CR conditions.

Of the four possible pathways for glyceraldehyde metabolism, the TK-catalysed one is the predominant pathway, since the TK enzyme has the lowest  $K_m$  of all other enzymes involved in glyceraldehyde metabolism [16]. Our present results indicate that CR significantly influenced TK activity only in old mice (Figure 1A), which is in agreement with a previous observation [17]. TK is an important enzyme, since it converts glyceraldehyde into GAP, therefore, completing the entry of fructose into the glycolytic pathway (Figure 4). Previous reports have shown increased TK activity in the presence of high dietary sucrose [38,39] or fructose [40], implying activity modulation by these sugars. These findings indicate that glucose supplementation alone did not change activity, and that the fructose moiety was the cause of the increased activity [38]. In contrast, one short-term (3 days) fructose-feeding study reported no increase in activity [41]. A wealth of information on ALDH concerning subjects as diverse as cellular distribution, developmental expression, carcinogenesis and others is already available [34,42,43]. However, to the

best of our knowledge, this information does not extend to the influence of caloric restriction on the activity of the enzyme. The lack of activity changes by CR in both young and old mice (Figure 1C) indicate that this pathway is not responsive to calorie intake and may not be important for the metabolism of glyceraldehyde. Moreover, no significant age differences were observed when young controls and CR were compared with their old counterparts. This lack of age difference is in agreement with previous findings from rat liver, where activities between 3-month-old and 23–26-month-old [44], and 3-month-old and adult [45], rats were similar.

Similar to ALDH, a vast amount of information is also available in the literature concerning ADH; however, none of this covers the influence of caloric restriction on the activity of this enzyme. ADH catalyses the reversible conversion of alcohols into their corresponding aldehydes and ketones. The trend towards a decrease ( $P = 0.08$ ) in our results (Figure 1B) between CR and control mice in both young and old groups could indicate a role for ADH in the glyceraldehyde into glycerol interconversion. On the other hand, the lack of age-related differences between young and old mice, in both control and CR groups (Figure 1B) is in agreement with previous work [46]. Previous studies reported



**Figure 4 Glyceraldehyde and glycerol metabolism and their interconnection to other metabolic pathways**

Enzymes investigated in the present study are indicated in underlined and italicized letters. Metabolites assayed were DHAP, G3P and glycerol. Bold arrows indicate the pathways studied. D-GlyK, D-glycerate kinase; F-1,6-BP fructose 1,6-bisphosphate; FFA, non-esterified fatty acid; F-1-P fructose 1-phosphate; F-6-P fructose 6-phosphate; G-6-P glucose 6-phosphate; LDH, lactate dehydrogenase; PDH, pyruvate dehydrogenase; 2-PG, 2-phosphoglycerate; TIM, triose phosphate isomerase; TG, triacylglycerol.

decreased ADH activity due to decreased dietary protein content (dietary protein restriction), rather than decreased calories [47–50]; however, increased dietary protein did not result in increased ADH activity [47]. This is of interest as the diet used in our present study to feed the CR group has a higher protein content than the diet of the control group [27], yet no increased ADH activity was observed in the CR mice.

ALR on the other hand, is one of several enzymes that form the aldo-keto reductase superfamily of enzymes, characterized by the NADPH-dependent reduction of a vast array of aldehydes [51–54]. To our knowledge, no studies could be found on the activity of ALR under various nutritional conditions or concerning the effects of caloric restriction on its activity. However, there are several studies available concerning the characteristics of ALR from various sources and its substrate specificities. The lack of significant differences in activity between CR and control mice in both young and old groups (Figure 1D) indicates that the pathway is not responsive to changes in calorie intake and may not be important for the metabolism of glyceraldehyde. Significant age differences between old and young mice, on both diets, are in agreement with the pattern found in rat liver [55].

The metabolism of glycerol is also of great interest, because, as a junctional metabolite, it occupies an important interconnecting position in metabolism. Also, in several species, it is an

important substrate for energy metabolism and biomass synthesis [6]. Similar to the enzymes involved in glyceraldehyde metabolism, information concerning the effects of caloric restriction on glycerol-metabolizing enzymes is lacking, although the effects of fasting for up to 24 h have been reported [56]. The significantly increased activities of GK (Figure 2A) and cG3PDH (Figure 2B) in both old and young CR mice is interesting, since glycerol is a gluconeogenic substrate [2,3] and caloric restriction induces sustained increases in gluconeogenesis [24,57], therefore, allowing glycerol to be utilized and converted into glucose. It has been shown that, during starvation, adipose tissue lipolysis gives rise to the release of glycerol and fatty acids into the blood, with the subsequent transport of glycerol to liver for further metabolism and entry into the glycolytic pathway [56]. Lipolysis occurs during caloric restriction [58,59], therefore, making glycerol available for metabolism in the liver by the increased activities of GK and cG3PDH. One striking phenotype observed in animals on a CR diet is the loss of fat mass [60] that results in lean animals, in which the liver plays a dominant role, as shown by increased gluconeogenesis and decreased glycolysis [23,24,57,61]. Additionally, increased levels of ketone bodies and transamination are also observed under CR conditions [23,24], indicating sustained capacities for utilization of fat and proteins respectively. It is interesting that increased gluconeogenic capacity with CR is maintained, even after much of the fat mass has disappeared and the animals are no longer undergoing dynamic weight loss.

The activity of mG3PDH in young and old CR mice was not significantly different from young and old controls (Figure 2C). Similar to cG3PDH, information concerning the effects of CR on mG3PDH activity is also lacking, and previous reports have also shown mG3PDH, but not cG3PDH, to be regulated by thyroid hormones [9,62]. It is known that thyroid hormone levels are decreased with caloric restriction [19,63], however, our present results suggest that this had no significant effect on mG3PDH activity in liver. It is possible to speculate that mG3PDH expression and activity in liver are very low, and, although mG3PDH with cG3PDH are part of the G3P shuttle, the malate–aspartate shuttle is the predominant shuttle in liver [1,4,11,64] and only cG3PDH is needed for the conversion of G3P into DHAP. Therefore, the need for a highly active mG3PDH does not exist in this tissue unlike its role in brain [8,64], skeletal muscle [9,64] and the flight muscle of insects [10,64].

Hepatic glycerol metabolism in mice has been reported to be regulated by PPAR $\alpha$  (peroxisome-proliferator-activated receptor  $\alpha$ ), the expression of which is increased by fasting (2.5–24 h) and leads to increased expression of glycerol metabolism genes, such as GK, cG3PDH, mG3PDH and the glycerol transporters aquaporins 3 and 9, with cG3PDH being the direct target for PPAR $\alpha$  [56]. In adipose tissue, on the other hand, glycerol metabolism is regulated by PPAR $\gamma$  [56], the nuclear receptor that promotes adipogenesis. In mammals under CR conditions, Sirt1 (sirtuin 1) is induced and promotes lipolysis and fat mobilization from adipose tissues into the blood, and represses PPAR $\gamma$  and adipogenesis [59,65,66]; therefore, glycerol becomes available for utilization as a source for glucose synthesis under caloric restriction. It has also been shown that expression levels of PPAR $\alpha$

and PPAR $\gamma$  were influenced by aging [67–69] and by caloric restriction [69]. It is possible that in our present study, the increased activities of GK and cG3PDH in both old and young CR mice were due to increased PPAR $\alpha$  expression. Moreover, PPAR $\alpha$  has also been reported to stimulate hepatic ketogenesis and  $\beta$ -oxidation [56,70], which is of interest as we have shown previously [23] that ketone body levels increase under CR conditions.

Plasma glycerol levels (Figure 3D) in both old and young CR mice were decreased, significantly in old CR ( $P < 0.045$ ) and showed a trend in young CR ( $P = 0.06$ ) mice. This decreased level of serum glycerol is probably due to increased activities of GK and cG3PDH. The breakdown of triacylglycerols during lipolysis results in glycerol generation, which is transported to the liver via the blood, where it is taken up for glucose synthesis through gluconeogenesis. Previously, it was shown that glycerol levels in fasted mice were lower, even though there was increased glycerol release from adipose tissue, and this was attributed to the fact that PPAR $\alpha$  activation resulted in increased expression of genes involved in hepatic gluconeogenesis (GK, cG3PDH etc.) from glycerol [56]. In the present study, we observed a decrease in glycerol levels in CR mice and increased GK and cG3PDH activities, therefore, it is possible that a similar mechanism is in operation.

Of the two hepatic metabolites (G3P and DHAP) measured (Figures 3A and 3B), G3P showed an increased pattern in both old and young CR mice (Figure 3A), matching that of GK (Figure 2A), indicating that the increase was probably due to the increased GK activity. In the case of DHAP levels, old CR mice showed a pattern of increase which matched that of cG3PDH; however, this was not the case for young CR where no change in DHAP level was observed between young CR and controls. This was probably due to the fact that the enzymes of the gluconeogenic pathway upstream of the DHAP $\rightarrow$ GAP conversion point (Figure 4) were unaffected by caloric restriction in young mice [23,24]. The two metabolites were used to determine the G3P/DHAP ratio, which reflects the cytoplasmic NADH/NAD ratio, in a manner similar to the lactate/pyruvate ratio. Increased NADH/NAD ratios were reported previously in animals under different dietary restriction conditions [23,71], indicating a more reduced state. Higher G3P/DHAP ratios in CR animals (Figure 3C) indicate a more reduced state, which is in agreement with a previous report [23]. Also, increased G3P levels are associated with decreased PFK-1 (6-phosphofructo-1-kinase) activity, since the metabolite is an allosteric inhibitor of PFK-1 [72]. Moreover, G3P inhibits PFK-2 (6-phosphofructo-2-kinase), which is responsible for F-2,6-BP (fructose 2,6-bisphosphate) synthesis, hence decreasing the intracellular levels of F-2,6-BP [72]. F-2,6-BP is a potent activator of PFK-1 and inhibitor of F-1,6-BPase (fructose 1,6-bisphosphatase) [73]. This is interesting, because our previous results have shown that, under CR conditions, decreased PFK-1 activity and F-2,6-BP levels [23] and increased F-1,6-BPase activity [24] were observed.

The results from our present study have shown that glycerol and glyceraldehyde metabolisms are influenced by sustained cal-

oric restriction. Information concerning the effects of caloric restriction on glyceraldehyde metabolism is almost non-existent and, with the exception of TK, none of the other enzymes showed any major changes under CR conditions, except for ADH which showed a trend towards decreased activity. Glycerol metabolism showed a much different pattern in that GK and cG3PDH were influenced by caloric restriction, whereas mG3PDH was not. The results indicate that long-term caloric restriction induces sustained increases in the capacity for gluconeogenesis from glycerol. We have discussed the role of PPAR in this regulation, and future studies are required to elucidate the mechanisms involved in the regulation of glyceraldehyde and glycerol metabolism under sustained CR conditions.

#### ACKNOWLEDGMENTS

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