Mechanisms of fructose-induced hypertriglyceridaemia in the rat

Activation of hepatic pyruvate dehydrogenase through inhibition of pyruvate dehydrogenase kinase

Ock-Jin PARK,*§ Denise CESAR,* Dennis FAIX,* Ken WU,* Cedric H. L. SHACKLETON† and Marc K. HELLERSTEIN*‡||

*Department of Nutritional Sciences, University of California, Berkeley, CA 94720,
†Oakland Children's Hospital Research Center, Oakland, CA 94609,
and ‡Department of Medicine, Division of Endocrinology and Metabolism, San Francisco General Hospital, University of California, San Francisco, CA 94110, U.S.A.

1. The effects of purified diets containing 70% glucose or 70% fructose on the activation state of hepatic pyruvate dehydrogenase (PDHa), activity of mitochondrial PDH kinase, plasma triacylglycerols (TG) and hepatic lipogenesis *de novo* in rats were measured. 2. Plasma TG were significantly increased in the fructose-fed compared with the glucose-fed group (125 ± 45 mg/dl versus 57 ± 19 mg/dl; $P < 0.002$) after 3–5 weeks on the diet despite less daily food intake. 3. Hepatic PDHa in fructose-fed rats was 144% of the value in glucose-fed rats (15.4 ± 1.2% versus 10.7 ± 0.5%; $P < 0.002$), whereas cardiac muscle PDHa was not different (45.5 ± 6.6% versus 41.0 ± 7.8%). 4. Intrinsic hepatic PDH kinase activity was decreased to 34% of glucose-fed values by fructose feeding ($-k = 3.56 \pm 0.39$ versus 10.41 ± 1.85 min^{-1}; $P < 0.005$). 5. The fractional contribution to very-low-density-lipoprotein palmitate from hepatic lipogenesis *de novo*, measured by a stable-isotope mass-spectrometric method, was 10.49 ± 2.42% ($n = 8$) in fructose-fed rats versus 5.55 ± 1.38% ($n = 9$) in glucose-fed rats ($P < 0.05$), and 2.66 ± 2.39% ($n = 3$) in chow-fed rats ($P < 0.05$ versus fructose-fed group). The absolute contribution to circulating TG from lipogenesis *de novo* was also significantly higher in the fructose-fed than in the glucose-fed group (14.9 ± 5.1 mg/dl versus 2.9 ± 0.6 mg/dl; $P < 0.05$) 6. Portal insulin concentrations were significantly higher in the fructose-fed rats (206 ± 49 μ-units/ml versus 81 ± 15 μ-units/ml; $P < 0.05$). 7. In conclusion, dietary fructose appears to have a specific activating effect on hepatic PDH, mediated at least in part by inhibition of PDH kinase. These results are consistent with increased flux through hepatic PDH and synthesis of new fat, not just increased re-esterification of non-esterified fatty acids.

INTRODUCTION

Dietary fructose exerts a number of adverse metabolic effects in experimental animals and in humans, including hypertriglyceridaemia [1–8], hyperinsulinaemia [4,6] and hypertension [9]. With dietary fructose consumption in the form of sucrose increasing in industrialized and developing countries [10], the potential public-health implications are important. The metabolic mechanisms underlying the effects of dietary fructose are not well understood. For hypertriglyceridaemia, most studies [2,4,7,8,11] have indicated that hepatic very-low-density-lipoprotein triacylglycerol (VLDL-TG) overproduction rather than impaired peripheral clearance is involved, although some have also suggested a role for decreased VLDL clearance [7,8]. In a general way, VLDL-TG overproduction is consistent with the initial metabolism of fructose being in the liver, in contrast with the initial metabolism of glucose, which is mainly extrahepatic [12,13], owing to the exclusively hepatic location of fructokinase, the lack of fructose phosphorylation by extrahepatic hexokinase in the presence of competing glucose, and the approx. 10-fold greater activity of hepatic fructokinase compared with combined hepatic glucokinase and hexokinase activities [14]. The general notion that, at equivalent energy loads, hepatic intermediary metabolism is more affected by ingestion of fructose than of glucose (exemplified by the greater anti-ketogenic effect of fructose [15–18]) does not differentiate between two fundamentally distinct metabolic mechanisms by which fructose might stimulate VLDL-TG synthesis, however.

Increased hepatic TG production might result from conversion of fructose carbon into new fat (lipogenesis *de novo*) or from increased esterification of circulating non-esterified fatty acids (NEFA) by the liver. Greater hepatic re-esterification of incoming NEFA is a plausible mechanism, in that fructose ingestion results in greater hepatic availability of pyruvate and α-glycerophosphate [19], hyper-insulinaemia [4,6], and might inhibit fatty acyl-CoA ester transport into mitochondria through increased malonyl-CoA availability [20]. This metabolic mechanism would not require synthesis of new fat. In contrast, hepatic conversion of fructose into new fat would require flux through hepatic pyruvate dehydrogenase (PDH) and synthesis of fat from the acetyl-CoA generated. Several authors have reported increased hepatic lipogenesis as a consequence of dietary fructose [11,19,21], but the effects of fructose on PDH remain uncertain.

The regulation of PDH activity is complex (for review, see Randle [22]), involving both substrate-level and phosphorylation–dephosphorylation mechanisms. Dephosphorylated PDH complex is the active form (PDHa). The fraction of PDH present as PDHa varies from tissue to tissue and is determined by the activities of PDH kinase (an intrinsic component of the mitochondrial PDH complex), PDH-phosphate phosphatase and the susceptibility of the substrate PDH to either enzyme [22,23]. Most studies of PDH regulation have been in the direction of decreased activity, i.e. adaptation to starvation or insulin-deficient diabetes [22]. These have shown roles for increased

Abbreviations used: PDH, pyruvate dehydrogenase; PDHa, active form of PDH; VLDL, very-low-density-lipoprotein; TG, triacylglycerols; NEFA, non-esterified fatty acids; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

§ Present address is: Department of Food and Nutrition, Han Nam University, 133 Ojung Dong Chung-Fu, Taejon, Korea.
|| To whom correspondence should be sent, at either address.

PDH kinase activity [22,24], as well as altered substrate concentrations in restricting PDH activity and pyruvate oxidation in these settings of hepatic carbohydrate depletion. An equivalent mechanistic understanding has not yet emerged in the direction of excess hepatic carbohydrate availability (e.g. dietary fructose).

Here, we address the mechanisms of fructose-induced hypertriglyceridaemia in the rat. We compare the effects of 70%-glucose or -fructose diets on plasma TG concentrations, portal insulin concentrations, hepatic and cardiac muscle PDHa, hepatic PDH kinase activity and hepatic lipogenesis *de novo*.

METHODS

Diet

Rats were housed in individual cages and maintained on a 12 h-light/12 h-dark cycle. The animals were fed *ad libitum* on a purified diet containing 70% fructose or glucose. These diets consisted of (by wt.) 70% sugar, 2% fibre, 18% vitamin-free casein (Teklad, Madison, WI, U.S.A.), 5% corn oil (Mazola), 3.5% Teklad Mineral Mix, 1% ICN vitamin mix (AIN vitamin mixture 76A), 0.3% L-methionine and 0.2% choline chloride. Some rats were also maintained *ad libitum* on ground Purina rat chow.

Chemical reagents

Sodium *p*-[(*p*-aminophenyl)azo]benzenesulphonic acid ('AABS') was from Pfaltz and Bauer (Waterbury, CT, U.S.A.). Digitonin, EGTA, DL-dithoithreitol, phenylmethanesulphonyl fluoride, ATP, pigeon liver acetone-dried powder, thiamin pyrophosphate, NAD^+, CoA, acetyl-CoA, benzamidine, tosyl-L-lysylchloromethane, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and pyruvic acid were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Sodium [2-^{13}C]acetate was purchased from Isotec Inc. (Miamisburg, OH, U.S.A.).

PDH assay on liver and cardiac muscle

Tissue PDH activity was determined as described elsewhere [24,25] by a two-step spectrophotometric procedure. Tissues were frozen *in situ* in pentobarbital-anaesthetized rats by using pre-cooled aluminium tongs. The time from surgical entry of the abdomen to freezing of liver was less than 10–15 s [25]. Livers appeared well perfused, and the left lobe was frozen *in situ* and excised. Then 2–4 ml of portal blood was withdrawn, the thorax was entered and the still-beating heart was frozen and removed. The frozen tissue (liver or heart muscle) was powdered under liquid N_2 with a porcelain mortar and pestle. The weighed portion (0.2 g for liver, 0.1 g for heart muscle) of the powdered frozen tissue was extracted, by using a Polytron, in the extraction buffer (1 ml for liver samples, 3 ml for heart samples). PDH activity was measured spectrophotometrically by coupling the formation of acetyl-CoA from pyruvate to the acylation of *p*-[(*p*-aminophenyl)azo]benzenesulphonic acid [24,25]. Total PDH activity was assayed after incubation of 40 μl of extracts with ox heart PDH-phosphate phosphatase (0.5 unit/ml) to dephosphorylate (activate) PDH present. PDH activity was then re-assayed after activation. A unit of enzyme activity converts 1 μmol of substrate into product/min at 30 °C [24]. PDHa is expressed as percentage active form, from the ratio of PDH activity before to that after dephosphorylation with PDH-phosphate phosphatase.

PDH kinase assay on liver mitochondria

For hepatic PDH kinase measurements, the right lobe of the liver was excised (after freeze-clamping of the left lobe *in situ*) and immediately placed in ice-cold homogenization buffer. In these animals, cardiac muscle PDHa was not measured. Preliminary studies showed that there were no differences between left and right lobes of liver for PDH kinase activity. Liver tissue (1–1.5 g) was disrupted in 0.25 M-sucrose containing 5 mM-Tris and 2 mM-EGTA, pH 7.5. After a low-speed spin (750 *g* for 10 min) to remove debris, the mitochondrial pellets were obtained by high-speed centrifugation (10000 *g* for 7 min). Mitochondria were resuspended in sucrose buffer containing digitonin (0.4 mg/ml), incubated for 2 min and centrifuged (600 *g* for 5 min) to remove lysosomal debris. Mitochondria were then pelleted (10000 *g* for 7 min), resuspended in sucrose buffer and re-centrifuged (10000 *g* for 7 min). Respiring mitochondria were incubated at 4 mg of protein/ml for 30 min in a medium containing 0.12 M-KCl, 20 mM-Tris/HCl, 5 mM-K_2HPO_4, 2 mM-EGTA and 10 μM-CCCP, pH 7.4. This procedure fully converts inactive PDH complex into active dephosphorylated complex (PDHa) by uncoupling oxidative phosphorylation and depleting mitochondrial ATP. Mitochondrial pellets were then separated by high-speed centrifugation and frozen in liquid N_2. Extracts were prepared by freezing and thawing three times in 30 mM-potassium phosphate/5 mM-EGTA/5 mM-dithiothreitol/0.3 mM-tosyl-lysylchloromethane/1 mM-benzamidine/1 mM-phenylmethanesulphonyl fluoride/1% (v/v) ox serum, pH 7.5 (72.5 mg of protein/ml). For assay of PDH kinase, 450 μl of extract containing 1 mM-$MgCl_2$ and 20 m-units of PDH complex was incubated for 3 min at 30 °C, and then 0.5 mM-ATP was added. At three or four time points (every 15 s after addition of ATP), samples were removed and added to PDH assay cuvettes. PDH kinase activity was calculated as the rate constant for ATP-dependent inactivation of PDH ($-k$), as described by Marchington *et al.* [24].

Blood metabolite measurements

Portal blood was obtained at the time of killing in lightly heparinized syringes and centrifuged at 4 °C. The plasma was stored at −20 °C before analyses. Plasma TG were measured by a glycerokinase assay technique (Sigma). Insulin was measured by radio-immunoassay. Glucose was determined by auto-analyser.

Isotopic studies

Chronic indwelling intrajugular silastic catheters were placed and maintained as described elsewhere [25], after $3\frac{1}{2}$–$4\frac{1}{2}$ weeks of purified diets or chow. Infusion studies were performed in groups of four rats (two glucose-fed, two fructose-fed) after animals returned to baseline food intake (48–96 h after catheterization). Sodium [2-^{13}C]acetate was infused at 0.020 mmol/kg per min from midnight; then at 8.00 h a constant infusion of sulfamethoxazole at 60 mg/kg per h was begun, following a priming dose of 60 mg/kg. The sulfamethoxazole is used as a trap or probe for hepatic acetyl-CoA, in order to confirm the isotopomer-calculated precursor enrichment for hepatic lipogenesis [27,28]. The true precursor enrichment is calculated by using a model of isotopomeric frequencies based on the binomial distribution [29]. Fractional lipogenesis *de novo* is then calculated. The enrichment of the acetate moiety of sulfamethoxazole acetate is measured by h.p.l.c./mass spectrometry [30]. The enrichment and isotopomer distribution pattern of VLDL-palmitate is measured by g.l.c./mass spectrometry [27].

Statistics

Groups were compared by ANOVA, using a two-tailed *t* test. Catheterized rats from glucose- and fructose-fed groups were paired at the time of catheterization and compared by paired *t* test for isotopic parameters. One glucose-fed rat did not have a paired fructose-fed animal and was not included in the paired *t*-test calculations.

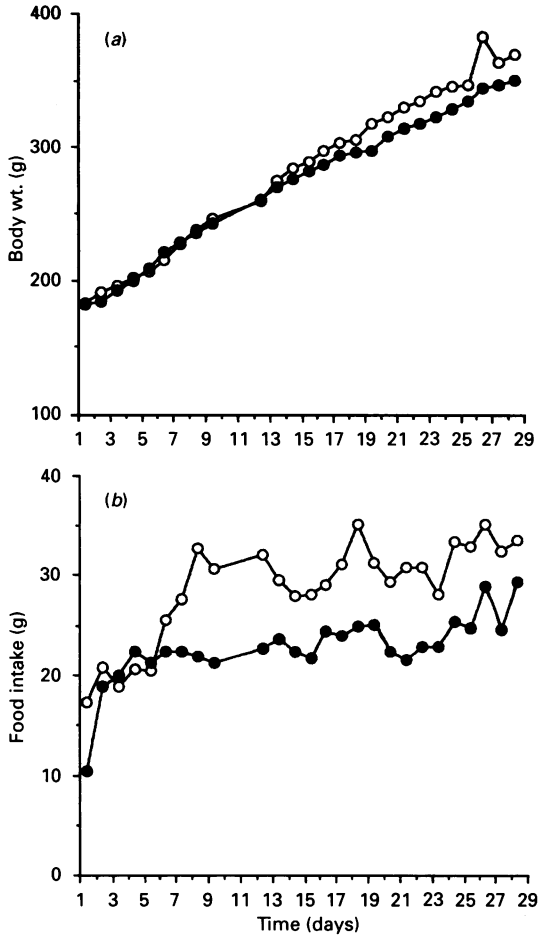

Fig. 1. Body weights (*a*) and daily food intake (*b*) in rats fed *ad libitum* maintained on either a 70%-glucose or 70%-fructose diet

○, Glucose-fed rats ($n = 12$). ●, Fructose-fed rats ($n = 12$). *$P < 0.01$ between groups. Rats were not weighed on days 10 and 11, so results for those days are not shown.

RESULTS

Food intake and weight gain

The average food intake and body weight of rats on the fructose- and glucose-based diets are shown (Fig. 1). Glucose-fed rats ate greater amounts of food (25–30 g/day) than did fructose-fed rats (20–25 g/day) ($P < 0.01$ between groups after day 8). Both groups gained weight in a steady manner (Fig. 1).

Plasma TG concentrations

Despite the lower food intake, rats fed on a fructose diet *ad libitum* exhibited over 2-fold elevated plasma TG concentrations (Table 1, $P < 0.005$).

PDHa in liver and cardiac muscle

The pattern for hepatic PDHa was similar to that of serum TGs (Table 1). PDHa was 44% higher in livers from fructose-fed rats compared with glucose-fed rats ($P < 0.002$). In contrast, there were no significant differences attributable to dietary carbohydrate source for cardiac muscle PDHa (Table 1). There were no significant differences between groups for total hepatic PDH activity in liver or cardiac muscle (Table 1).

Hepatic PDH kinase activity

Intrinsic PDH kinase activity was determined on extracts of isolated hepatic mitochondria (Table 1). Kinase activity is expressed as the rate constant ($-k$) for ATP-dependent inactivation of PDH which had previously been fully activated (dephosphorylated) by incubation of mitochondria with CCCP (see above and [24]). Hepatic PDH kinase activity in fructose-fed rats was decreased to 34% of that in glucose-fed rats ($P < 0.005$). The lower hepatic PDH kinase activity is consistent with, and may explain, the higher percentage of active (less phosphorylated) PDH present in the livers of these rats (see above).

Portal insulin concentrations

Portal insulin concentrations were significantly higher in fructose-fed rats than in glucose-fed rats ($P < 0.05$; Table 1).

Lipogenesis *de novo*

The fractional contribution of the '*de novo*' lipogenic pathway to circulating VLDL-palmitate was measured by a recently described stable-isotopic technique [27,28]. The contribution from lipogenesis *de novo* was 89% higher in fructose-fed rats than in glucose-fed rats (Table 1; $P < 0.05$). The fructose values are also significantly higher than values measured in three rats chow-fed *ad libitum* ($2.66 \pm 2.39\%$; $P < 0.05$). Because hepatic re-esterification of NEFA is also likely to be increased by fructose feeding [4,6,19,20], changes in the fractional contribution from the '*de novo*' pathway to circulating TG may underestimate the absolute increase in lipogenesis *de novo*. Accordingly, fractional contribution from the '*de novo*' pathway was multiplied by the circulating TG concentration to calculate the concentration of circulating TG attributable to lipogenesis *de novo* (Table 1). This calculation assumes that the average

Table 1. Comparisons between glucose- and fructose-fed groups

Feeding protocols and analytical techniques are described in the text. Measurements were made after 4–5 weeks of dietary glucose or fructose. Values shown are means ± S.E.M. for the numbers of observations in parentheses: *$P < 0.05$, **$P < 0.005$, ***$P < 0.001$ versus fructose-fed group. Total PDH present in tissues (units/mg wet wt. of tissue) did not differ between groups [cardiac muscle: fructose-fed, 3.84 ± 0.19 ($n = 10$), glucose-fed, 3.90 ± 0.64 ($n = 12$); liver: fructose-fed, 1.88 ± 0.26 ($n = 4$); glucose-fed, 1.91 ± 0.14 ($n = 10$)]. Abbreviation used: $-k$, rate constant for ATP-dependent inactivation of PDH (min^{-1}).

Group	Serum [TG] (mg/dl)	Portal insulin (μ-units/ml)	Cardiac muscle PDHa (%)†	Hepatic PDHa (%)†	Hepatic PDH kinase ($-k$)	Fractional lipogenesis *de novo* (%)‡	[TG] derived from lipogenesis *de novo* (mg/dl)
I Fructose-fed	125 ± 45 (12)	206 ± 49 (12)	45.5 ± 6.6 (10)	15.4 ± 1.2 (9)	3.56 ± 0.39 (6)	10.49 ± 2.42 (8)	14.9 ± 5.1 (8)
II Glucose-fed	57** ± 19 (11)	81* ± 15 (9)	41.0 ± 7.8 (12)	10.7*** ± 0.5 (10)	10.41** ± 1.85 (6)	5.55* ± 1.38 (9)	2.9* ± 0.6 (9)

† % refers to the fraction of cardiac or hepatic PDH in the active form.
‡ % refers to the fraction of VLDL-palmitate derived from lipogenesis *de novo*.

chain length of fatty acid newly synthesized by rat liver is C_{16} [31]. In fructose-fed rats, this parameter was more than 5-fold greater than in the glucose-fed group ($P < 0.05$). These results are consistent with previous reports of accelerated lipogenesis *de novo* in fructose-fed rats studied by the ^3H_2O-incorporation technique [11,19,21].

DISCUSSION

Pyruvate oxidation at the PDH step is irreversible and tightly regulated. The effects of substrate deprivation have been carefully studied [22–24]. Prolonged fasting decreases pyruvate oxidation [22,25]; PDHa decreases [22,24,25] concurrently with activation of PDH kinase [24]. The increase in PDH kinase activity in turn may be due to the emergence of a 'stable' inhibitory factor (i.e. not a circulating substrate, hormone or other factor that might change rapidly with refeeding), initially termed 'kinase activator protein' [22,24], although more recently the existence of a separate protein has been called into question [26]. The experimental imposition of carbohydrate deprivation has thereby provided a useful model for investigating potentially new regulatory mechanisms that control PDH activity and pyruvate disposal. A similar understanding is not currently available in the direction of overfeeding (hepatic carbohydrate excess). We reasoned that fructose-induced hypertriglyceridaemia might be a simple model of carbohydrate excess and asked whether changes in PDHa, PDH kinase or lipogenesis would be the mirror image of adaptations to substrate deprivation, or whether different regulatory mechanisms would need to be invoked.

Our data clearly indicate that hepatic PDH activity is higher (approx. 50% increased; Table 1) and PDH kinase activity is lower (decreased by two-thirds) in rats chronically fed on fructose compared with glucose-containing diets. These changes paralleled higher serum TG concentrations, portal insulin concentrations and lipogenesis *de novo* (Table 1) in the fructose-fed group, despite lower daily food intakes (Fig. 1) and no difference in extra-hepatic (cardiac muscle) PDHa (Table 1). The hepatic effects of dietary fructose cannot therefore be non-specifically due either to excess total energy or excess carbohydrate energy, but must be specific effects of fructose rather than glucose on the liver. The effects of dietary fructose on serum TG concentrations and liver metabolism have previously been shown to change over time [7,32], so we maintained our animals on the diets for 4–5 weeks. We were still able to see clear differences between the two carbohydrate-based diets, suggesting that the effect is persistent.

Previous studies of chronic dietary fructose effects on hepatic PDH are not definitive. Several authors [33–35] have described the acute effects of fructose on hepatic metabolism, including PDH phosphorylation state. The mechanism of these acute fructose effects is ATP depletion with secondary PDH dephosphorylation, whereas chronic fructose diets do not cause hepatic ATP depletion [32]. Carmona & Freedland [21] reported that a 60%-fructose diet doubled ^3H_2O incorporation into fat by isolated hepatocytes and specifically made lactate a better lipogenic substrate, whereas acetate or butyrate were the preferred substrates in glucose-fed rats. They suggested that activation of PDH was involved, but did not measure PDH activity. Others [14,19] have measured the activities of hepatic malic enzyme, citrate-cleavage enzyme and acetyl-CoA carboxylase (the first two being increased by dietary fructose [19]), but did not measure PDH. The only published study of hepatic PDH in chronic fructose feeding [36] is technically flawed. Whole liver extracts were used with a [1-^{14}C]pyruvate-to-$^{14}CO_2$ radiometric assay, which can lead to radioisotopic artifacts in liver owing to the activity of other decarboxylating enzymes (phosphoenolpyruvate carboxykinase and tricarboxylic-acid-cycle enzymes) as noted by Myles *et al.* [37]. Also, there was no attempt to activate PDH *in vitro* in order to measure percentage of active form, so that only crude activities were presented. Although our finding of elevated hepatic PDHa is consistent with the physiological studies noted [11,19,21], this did not have to be the case; substrate-level control in principle might have mediated increased flux across PDH without a change in phosphorylation state (see below).

To our knowledge, this is the first demonstration of a physiological regulatory role for PDH kinase in the overfeeding direction. It will be of interest to search for the mechanism of PDH kinase inhibition, by analogy with PDH kinase activation in prolonged fasting [22,24]; e.g. is there a kinase inhibitory protein or loss of a kinase activator?

The signals for inhibition of hepatic PDH kinase and activation of hepatic PDH by dietary fructose remain unknown. The lack of an increase in cardiac muscle PDHa suggests that the signal is intra-hepatic rather than systemic (i.e. not explained by circulating insulin alone). Although work in Bergman's laboratory [38] has suggested that fructose increases the 'efficiency' of glucose 6-phosphate conversion into glycogen rather than providing the substrate for glycogen deposition (their calculations indicated a redundancy in glucose 6-phosphate supply), our findings suggest that a net addition of glycolytic intermediates results from chronic fructose ingestion and that some of the additional flux ends up traversing hepatic PDH and entering fat stores. More than just inhibition of phosphorylase *a* [39] and glycogenolysis [38] must be involved, although the added carbon may be derived either from fructose itself or from the enhanced phosphorylation of glucose by glucokinase in the presence of fructose [40].

Although an increase in carbon flux through PDH may be inferred from our results, this need not be the only mechanism by which chronic fructose feeding increases VLDL-TG production and circulating TG levels. Elevated hepatic α-glycerophosphate concentrations, increased serum insulin (Table 1) or other mechanisms (e.g. increased malonyl-CoA in association with increased lipogenesis [20]) might encourage hepatic re-esterification rather than oxidation of NEFA. It is well established that fructose has an inhibitory effect on ketogenesis and a stimulatory effect on NEFA re-esterification in systems ranging from isolated hepatocytes [15], liver slices [16] and perfused liver [17,41] to diabetic human subjects [18]. VLDL-TG could be increased in this manner, but a net increase in body fat could not result from the re-esterification mechanism alone; adipose fat would still ultimately be derived from dietary fat. Our results with PDHa, PDH kinase and lipogenesis *de novo* suggest that new body fat is a metabolic consequence of dietary fructose, even in the absence of true overfeeding (Fig. 1). The effect observed here might be exaggerated in the presence of excess total energy, as would often be the case with sucrose-containing human diets. Lipogenesis *de novo* from carbohydrate is usually a minor pathway quantitatively in humans [27] and rats [42] under non-overfed conditions: prolonged and massive carbohydrate overfeeding is apparently required to stimulate lipogenesis *de novo* in humans [43]. Fructose feeding might therefore represent a physiological condition without positive energy balance where lipogenesis *de novo* has an important functional role. Quantitative pathway fluxes need to be measured to answer this question properly, however. Also, the relevance of 70%-fructose diets in animal models to the overconsumption of sucrose or fructose by human populations remains to be established. Nevertheless, it is of interest that moderate dietary fructose loads in humans have both acute effects (greater thermogenesis [44,45]) and chronic effects (hypertriglyceridemia), which might be explained, at least in part, by stimulation of lipogenesis *de novo*. This question can

be directly addressed in humans by using the stable-isotope method described here [27].

It is also worth emphasizing that an increase in carbon flux through PDH and into lipogenic pathways did not necessarily imply a change in PDHa. Non-covalent regulation of PDH by metabolites acting indirectly as modulators of PDH activity or directly by altering concentrations of substrates or products would have been reasonable mechanisms for increasing flux through the enzyme [22]. We have recently found [46] that recombinant cytokines (tumour necrosis factor and interleukin-1) markedly stimulate hepatic lipogenesis *de novo* in fed rats *ad libitum* despite a relatively minor effect on PDHa and no effect on PDH kinase activity. The relationship between PDH phosphorylation state and flux *in vivo* through the enzyme needs to be established separately under different metabolic conditions.

In summary, chronic dietary fructose feeding results in a 44% increase in the fraction of hepatic PDH in the active dephosphorylated form (PDHa), which may be explained by a 66% decrease in the activity of intrinsic mitochondrial PDH kinase. These enzymic changes are consistent with an observed increase in lipogenesis *de novo*. Dietary fructose may contribute to the observed increase in circulating TGs by contributing new fatty acid molecules as well as by signalling a re-esterifying anti-ketogenic state for liver fatty acid disposal. Thus the adaptation by hepatic PDH to abundant hepatic carbohydrate energy appears to be an extension of the adaptation to carbohydrate deprivation, namely modulation of PDH covalent activation state by PDH kinase. Whether PDH-phosphate phosphatase activity is also modulated, whether dietary sucrose effects differ from those of fructose and whether fructose-induced hypertriglyceridaemia in humans is associated with increased synthesis of new fat are some of the remaining questions that can be addressed by using this dietary model.

We thank Laura Glines, Dr. Ronald Krauss and Angie DeCarlo for technical help, Cynthia Ho for care of the animals, Dr. Thomas Vary for help in initially setting up the PDH assay, Mark Hudes for statistical consultation, and Cici Hyde and Karen Buchanan for preparation of the manuscript. These studies were supported in part by grant DK 40995 from the National Institutes of Health (N.I.H.), BRSG grant 87-51 from the University of California at Berkeley (M.K.H.) and grant DK34400 and Shared Instrumentation Grant RR03300 from the N.I.H. (C.H.L.S.).

REFERENCES

1. McDonald, I. (1966) Am. J. Clin. Nutr. **18**, 369–378
2. Nestel, P. P., Carroll, K. F. & Havesten, N. (1970) Metab. Clin. Exp. **19**, 1–18
3. Bar-On, H. & Stein, Y. (1968) J. Nutr. **94**, 95–105
4. Zavaroni, I., Chen, Y.-D. I & Reaven, G. M. (1982) Metab. Clin. Exp. **31**, 1077–1083
5. Nikkila, E. A. & Ojala, K. (1965) Life Sci. **4**, 937–943
6. Selder, J., Chen, Y.-D., Cully, M. D. & Reaven, G. M. (1980) Metab. Clin. Exp. **29**, 303–305
7. Hirano, T., Mamo, J., Poapst, M. & Steiner, G. (1988) Am. J. Physiol. **255**, E236–E240
8. Waddell, M. & Fallon, H. J. (1973) J. Clin. Invest. **52**, 2725–2731
9. Reaven, G. M. (1988) Diabetes **37**, 1595–1607
10. Anderson, T. A. (1982) Annu. Rev. Nutr. **2**, 113–132
11. Boogaerts, J. R., Malone-McNeal, M., Archambault-Schexnayder, J. & Davis, R. A. (1984) Am. J. Physiol. **246**, E77–E83
12. Katz, J. & McGarry, J. D. (1984) J. Clin. Invest. **74**, 1901–1909
13. Radziuk, J., McDonald, T. J., Rubenstein, D. & Dupre, J. (1978) Metab. Clin. Exp. **27**, 657–669
14. Zakin, D., Pardini, R., Herman, R. H. & Sauberlich, H. E. (1967) Biochim. Biophys. Acta **144**, 242–251
15. Ontko, I. A. (1972) J. Biol. Chem. **247**, 1788–1800
16. Quastel, J. H. & Wheatley, H. M. (1933) Biochem. J. **27**, 1753–1762
17. McGarry, J. D. & Foster, D. W. (1971) J. Biol. Chem. **246**, 6247–6253
18. Dietze, G., Wicklmayr, M. & Mehnert, H. (1978) Diabetes **27**, 709–714
19. Chevalier, M. M., Wiley, J. H. & Leveille, G. A. (1972) J. Nutr. **102**, 337–342
20. McGarry, J. D. & Foster, D. W. (1980) Annu. Rev. Biochem. **49**, 395–420
21. Carmona, A. & Freedland, R. A. (1989) J. Nutr. **119**, 1304–1310
22. Randle, P. J. (1986) Biochem. Soc. Trans. **14**, 799–806
23. Pettit, F. H., Roche, T. E. & Reed, L. J. (1972) Biochem. Biophys. Res. Commun. **49**, 563–571
24. Marchington, D. R., Kerbey, A. L., Giardina, M. G., Jones, A. E. & Randle, P. J. (1989) Biochem. J. **257**, 487–491
25. Kaempfer, S., Blackham, M., Christiansen, M., Wu, K., Cesar, D., Vary, T. & Hellerstein, M. K. (1991) Am. J. Physiol. **260**, E865–E875
26. Mistry, S. C., Priestman, D. A., Kerbey, A. L. & Randle, P. J. (1991) Biochem. J. **275**, 775–779
27. Hellerstein, M. K., Christiansen, M., Kaempfer, S., Kletke, C., Wu, K., Reid, J. S., Hellerstein, N. S. & Shackleton, C. H. L. (1991) J. Clin. Invest. **87**, 1841–1852
28. Hellerstein, M. K., Wu, K., Kaempfer, S., Kletke, C. & Shackleton, C. H. L. (1991) J. Biol. Chem. **266**, 10912–10919
29. Hellerstein, M. K. (1991) J. Biol. Chem. **266**, 10920–10924
30. Reid, J. S., Wu, K., Kaempfer, S., Hellerstein, M. & Shackleton, C. (1990) Biomed. Mass Spectrom. **19**, 535–540
31. Foster, D. W. & Bloom, B. (1963) J. Biol. Chem. **238**, 888–892
32. Hill, P. (1969) Lipids **5**, 621–627
33. Mayes, P. A. & Laker, M. E. (1986) Prog. Biochem. Pharmacol. **21**, 33–58
34. Siess, E. A. & Wieland, O. H. (1976) Biochem. J. **156**, 91–102
35. Söling, H. D. & Bernhard, G. (1971) FEBS Lett. **13**, 201–203
36. Vrána, A., Rawlin, J., Loriette, C. & Razdova, L. (1983) Nutr. Rep. Int. **28**, 1437–1445
37. Myles, D. D., Strong, P. & Sugden, M. C. (1984) Biochem. J. **218**, 997–998
38. Youn, J. H., Kaslow, H. R. & Bergman, R. N. (1987) J. Biol. Chem. **262**, 11470–11477
39. Van den Berghe, G. (1978) Curr. Top. Cell. Regul. **13**, 97–135
40. Davies, D. R., Detheux, M. & Van Schaftingen, E. (1990) Eur. J. Biochem. **192**, 283–289
41. Topping, D. L. & Mayes, P. A. (1972) Biochem. J. **126**, 295–311
42. Patkin, J. K. & Masoro, E. J. (1964) Can. J. Physiol. Pharmacol. **42**, 101–107
43. Acheson, K. J., Schutz, Y., Bessard, T., Anantharaman, K., Flatt, J.-P. & Jéquier, E. (1988) Am. J. Clin. Nutr. **48**, 249–247
44. Simonson, D. C., Tappy, L., Jéquier, E., Felber, J.-P. & DeFronzo, R. A. (1988) Am. J. Physiol. **254**, E201–E207
45. Schwarz, J.-M., Schutz, Y., Froidevaux, F., Acheson, K.-J., Jeanprête, N., Schneider, H., Felber, J.-P. & Jéquier, E. (1989) Am. J. Clin. Nutr. **49**, 667–674
46. Blackham, M., Cesar, D., Park, O.-J., Vary, T. C., Wu, K., Kaempfer, S., Shackleton, C. H. L. & Hellerstein, M. K. (1992) Biochem. J., in the press

Received 30 August 1991/8 October 1991; accepted 22 October 1991