Effects of recombinant monokines on hepatic pyruvate dehydrogenase, pyruvate dehydrogenase kinase, lipogenesis *de novo* and plasma triacylglycerols

Abolition by prior fasting

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1. The effects of recombinant human tumour necrosis factor α (TNF) and murine interleukin -1 α (IL-1) on the activation state of the hepatic pyruvate dehydrogenase complex (PDHa), the activity of mitochondrial PDH kinase, hepatic lipogenesis *de novo* and plasma triacylglycerol (TG) concentrations were studied. 2. Monokine effects depended upon prior nutritional state. In rats fasted for 20 h or 45 h before monokine administration and refeeding (orally or with intravenous glucose), PDHa, TG and hepatic lipogenesis were not increased. In rats fed *ad libitum*, treatment with TNF plus IL-1 increased the contribution of hepatic lipogenesis to circulating TG to 550 % of control values ($P = 0.03$) and plasma TG concentrations to 159 % ($P = 0.02$), whereas PDHa increased slightly to 120 % ($P = 0.02$) and liver glycogen content fell to 45.8 % ($P = 0.05$) of control values. 3. Intrinsic hepatic PDH kinase activity was not changed by monokine treatment in rats fed *ad libitum*. 4. The increased lipogenesis *de novo* showed no correlation ($r^2 = 0.05$, not significant) with hepatic PDHa in individual animals fed *ad libitum*. 5. In conclusion, these results suggest that monokines increase pyruvate flux through hepatic PDH *in vivo* in rats fed *ad libitum* primarily by mechanisms other than covalent modification of PDH. Prior nutritional status exerts a permissive effect for monokine stimulation of PDHa and lipogenesis, consistent with a substrate-mediated action, but the mechanism of this permissive effect remains uncertain.

INTRODUCTION

Monokines secreted by stimulated monocytes/macrophages, such as interleukin-1 (IL-1), tumour necrosis factor (TNF) and interleukin-6 (IL-6), are believed to mediate most of the physiological alterations associated with acute and chronic infections [1,2]. Several metabolic disturbances present in infectious diseases have been reproduced by administration of recombinant monokines. Lactic acidosis is often present in sepsis [3] and is a notable consequence of large doses of TNF in rodents [2,4]. Hypoglycaemia and hyperglycaemia have both been observed after monokine administration [5]. Hypertriglyceridaemia is commonly observed in neoplastic conditions [6,7] and in bacterial [8], viral [9] or parasitic [10] infections; increases in the concentration of circulating triacylglycerols (TG) also result from administration of IL-1, TNF or interferon- γ in rodents and humans [11–15]. Initially, cytokine-induced hypertriglyceridaemia was believed to be due to decreased activity of lipoprotein lipase, leading to impaired clearance of very-low-density lipoprotein (VLDL)-TG [15]. More recently [11,12], stimulation of hepatic lipogenesis *de novo* and VLDL-TG production has been shown to be at least as important a mechanism of monokine-induced hypertriglyceridaemia. Finally, a significant metabolic conse-

quence of chronic infectious or inflammatory conditions is wasting of lean tissue. Disease-associated wasting is characterized by greater losses of lean tissue relative to fat stores as compared with simple fasting [16–18] and by the absence of certain nitrogen-sparing metabolic adaptations to the hypoenergetic state, such as ketosis [19,20].

The biochemical mechanisms behind cytokine-induced metabolic alterations remain mostly unknown. Of interest is the possible role of the pyruvate dehydrogenase (PDH) complex. PDH catalyses the irreversible removal of potentially gluconeogenic substrates in animal tissues, and as such is tightly regulated in hypoenergetic states [21,22]. Inappropriate activity of hepatic PDH might underlie the seemingly maladaptive increase in malonyl-CoA [20] and lipogenesis *de novo* [11,12] induced by infections or recombinant cytokines, as well as any failure to decrease pyruvate oxidation and conserve protein [16–18]. Conversely, decreased PDH activity might contribute to lactic acidosis seen in more serious acute infections or after sublethal doses of recombinant monokines [2,3]. The activity of the PDH complex is regulated by both end-product inhibition and phosphorylation/dephosphorylation (reviewed in [22]). The complex is inactivated by phosphorylation, which is catalysed by an intrinsic PDH kinase, and activated by dephosphorylation,

Abbreviations used: TNF, tumour necrosis factor; IL, interleukin; rh, recombinant human; rm, recombinant murine; PDH, pyruvate dehydrogenase complex; PDHa, percentage of active form of PDH; TG, triacylglycerols; VLDL, very-low-density lipoprotein; SMX, sulfamethoxazole.

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catalysed by a PDH-phosphate phosphatase. These regulatory activities in turn are modulated by intramitochondrial metabolite and cation concentrations [22,23]. In principle, then, changes in pyruvate flux through PDH might be mediated by changes in PDH kinase or phosphatase activities or by direct metabolite effects.

Evidence concerning the effects of recombinant monokines on hepatic PDH or its regulatory components is lacking. Here, we report the effects of administration of recombinant TNF α and IL-1 α *in vivo* on rat liver PDH activation state and mitochondrial PDH kinase activity. Because changes in PDH activity *in vivo* do not necessarily parallel changes in PDH phosphorylation state [22], we also measured hepatic lipogenesis *de novo* [24,25] as one index of pyruvate carbon flux through PDH. The influence of nutritional state on monokine-induced changes in these parameters is also addressed.

MATERIALS AND METHODS

Chemical and reagents

The reagents for performing the PDH assay and the PDH kinase assay are as described previously [26,27]. Cetus Corp. (Emeryville, CA, U.S.A.) generously provided recombinant human (rh) TNF α . The bioactivity is 24×10^6 units/mg. Endotoxin level is 0.02 ng/ml as determined by *Limulus* assay. Recombinant murine (rm) IL-1 α was kindly supplied by Dr. Peter LoMedico of Hoffman-LaRoche (Nutley, NJ, U.S.A.). The activity of the rmIL-1 α is 1.3×10^8 units/mg by assay with D₁₀-G4.1 cells [28], where 1 Lymphocyte Activating Factor unit = 20–50 D₁₀ units. The endotoxin concentration is 2.5 units/ml by *Limulus* assay. Sterile ibuprofen for parenteral administration was provided by the Upjohn Co. (Kalamazoo, MI, U.S.A.). Sodium [2-¹³C]acetate was from Isotec Inc. (Miamisburg, OH, U.S.A.). [2-¹⁴C]Acetate was purchased from ICN Radiochemicals (Irvine, CA, U.S.A.).

Assay for PDH activity

We measured PDH complex activity in tissue extracts by an indirect spectrophotometric method [26,29], wherein the formation of acetyl-CoA from pyruvate is coupled to the acetylation of a dye [*p*-(*p*-aminoazo)benzenesulphonic acid], using the enzyme arylamine acetyltransferase [29], which is prepared from pigeon liver [26]. The percentage of tissue PDH in the active dephosphorylated form (PDHa) is determined by incubation with PDH-phosphate phosphatase, prepared from bovine heart [26]. Sample preparation and the assay technique are described elsewhere [26].

Assay for PDH kinase activity

The activity of intrinsic PDH kinase in mitochondria isolated from liver was determined as described previously [27], in accordance with the method of Marchington *et al.* [29].

Measurement of hepatic lipogenesis *de novo* by using stable isotopes and radioisotopes

The fractional synthesis of circulating VLDL-fatty acids from the pathway of lipogenesis *de novo* was measured by using a new isotopic method based on the precursor-product relationship [24,25]. Stable isotopic enrichment or specific radioactivity of hepatic cytosolic acetyl-CoA (the true precursor) was determined by using the acetylated-xenobiotic probe technique [24,26]. Sodium [2-¹³C]acetate was infused intravenously at

0.020 mmol/h per kg, or [2-¹⁴C]acetate at 10 μ Ci/h, and sulfa-methoxazole (SMX) was infused as a 40–60 mg/kg intravenous bolus, followed by 40–60 mg/h per kg. Measurement of enrichment or specific radioactivity of circulating VLDL-fatty acids and the model and calculations have been discussed elsewhere [25,30]. TG content in serum was measured by a standard glycerokinase technique (Sigma Chemical Co., St. Louis, MO, U.S.A.) before and after enzymic hydrolysis to glycerol. M.s. analyses have been described previously [24,31], h.p.l.c./m.s. being used to measure enrichments of the precursor (SMX acetate) and g.l.c./m.s. for the enrichment of the product (VLDL-palmitate). For radioisotopic studies, the specific radioactivity of SMX acetate isolated from urine by h.p.l.c. [25] is determined by quantification (comparison of h.p.l.c. peak heights with authentic SMX-acetate standards) and liquid-scintillation counting (Beckman model LS6000). The acetate moiety of SMX-acetate represents 1/4.92 of the weight of the conjugate, so this correction factor is used for calculating the specific radioactivity (S.A.) of the acetate. The errors of both the counting and the quantifying steps are less than 1%, and the overall error of the estimate is less than 2% [25,26]. Authentic SMX acetate, kindly given by Hoffman-La Roche, was used to confirm the retention time and for quantification. Plasma VLDL was prepared by ultracentrifugation [24]. Before isolation of VLDL from animals refed orally, chylomicrons were removed by a 30 min spin at 47000 *g* at 17 °C (Beckman model L2-65B ultracentrifuge). Removal of non-covalently bound [¹⁴C]acetate from the VLDL fraction was carried out as described previously [25], by using heparin (1.0 unit) and MnCl₂ (2 M) to precipitate VLDL free of unincorporated [¹⁴C]acetate. The fractions of ¹⁴C radioactivity in the fatty acid and glycerol moieties of VLDL were determined by trans-methylation of VLDL-TG in methanolic HCl [25], followed by hexane extraction and counting of radioactivity of samples from the hexane-extractable and non-extractable solutions. Total VLDL-TG radioactivity is corrected for ¹⁴C radioactivity in the glycerol moiety to calculate radioactivity in fatty acids.

Hepatic lipogenesis *de novo* (%) was calculated with radioisotopes [25] as:

$$\frac{\text{S.A. VLDL-FA (d.p.m./}\mu\text{g of C)}}{\text{S.A. acetate (SMX) (d.p.m./}\mu\text{g of C)}} \times 100$$

where d.p.m./ μ g of C is calculated from acetate S.A. by dividing by 0.40 (carbon/total molecular mass) and from VLDL-TG S.A. by dividing by 0.715 [fatty acid (FA) carbon/molecular mass of TG, assuming glycerol tripalmitate]. Hepatic lipogenesis *de novo* with stable isotopes was calculated as described elsewhere [24,25]. The concentration of circulating TG attributable to lipogenesis *de novo* was calculated by multiplying the fractional contribution from lipogenesis *de novo* by the concentration of plasma TG [27].

Animals and experimental protocols

Male Sprague-Dawley rats were housed in individual cages at 22 °C with a 12 h-light/12 h-dark cycle (light 07:00–19:00 h). The diet consisted of Purina rat chow. Water was provided *ad libitum*. Some animals were fed on chow *ad libitum*, whereas others were trained to a single daily 3 h meal from 07:00 to 10:00 h (meal-fed) [32]. Meal-feeding is useful because it is a way of ensuring that the actual nutritional state of the animal is known at a given time and because food intake is compressed to a brief time interval, so that the fasted-to-fed transition can be reliably induced by the oral route over a relatively short experimental period. Studies on animals fed *ad libitum* were performed in the morning (07:00–8:00 h).

Chronic indwelling intrajugular silastic catheters for infusion studies were inserted as described previously [33]. Surgery was

performed under Metofane (methoxyflurane) anaesthesia, and animals were allowed to return to baseline food intake (24–48 h after catheterization) before further studies were performed. We have shown elsewhere that chronic catheterization does not alter hepatic PDHa in fasted or fed states [26]. Animals were killed by intraperitoneal or intravenous administration of pentobarbital (50 mg/kg), followed by laparotomy and freeze-clamping *in situ* of the right lobe of the liver for PDH activity measurements. The time from abdominal incision until freeze-clamping is less than 15 s, and good liver perfusion is maintained. The left lobe of the liver is then excised and mitochondria are isolated for measurement of PDH kinase activity. Portal blood is then withdrawn by syringe and the animals are exsanguinated. Recombinant monokines were mixed in 1 ml of 0.1% heat-inactivated (56 °C, 20 min) serum in 0.9% NaCl (vehicle). Control animals received vehicle intraperitoneally. Animals treated intraperitoneally with cytokines daily for tachyphylaxis studies were accustomed to injections by receiving daily vehicle injections for several days before starting cytokine administration. Injections were given at the same time each day, by the same person. Some animals were refed intravenously with glucose prepared under sterile conditions and administered at a rate of 25–30 mg/min per kg for 2–5 h before death. Liver glycogen content was determined in liver freeze-clamped *in situ* as described previously [33]. Plasma glucose was measured by a glucose oxidase technique. Serum insulin was measured by r.i.a. (Cambridge Medical Diagnostics, Billerica, MA, U.S.A.). The University of California at Berkeley Animal Uses Committee gave prior approval for all studies.

Statistics

Comparison of groups for differences was made by unpaired *t* test, at the 0.05 significance level. Group comparisons for parameters not involving lipogenesis *de novo* (PDHa, PDH kinase, liver glycogen, portal insulin, plasma glucose) used data from uncatheterized rats, whereas lipogenesis calculations were with data from catheterized rats.

RESULTS

Effects of rhTNF α on hepatic PDHa in chronically meal-fed rats after a 20 h fast

Rats maintained on a chronic meal-feeding regimen were given rhTNF α at doses from 25 to 250 μ g/kg intraperitoneally 1 h before their daily meal, i.e. after 20 h of their usual 21 h interprandial fast. Livers were sampled 2 h after the onset of meal-feeding. Total hepatic PDH activity in control rats (not given TNF) was 1.66 ± 0.20 (mean $\pm$ S.E.M.) units/g of tissue and was not significantly altered in any TNF-treated group. At TNF doses ranging from 25 to 250 μ g/kg, hepatic PDHa was not significantly different from control values (Table 1). Although the trend was for a somewhat decreased PDHa at doses of TNF between 25 and 100 μ g/kg, none of these differences reached statistical significance ($P = 0.29$ for overall ANOVA). Food intake in these animals is also shown (Table 1). All doses of TNF caused an acute difference in food intake. Rats pair-fed to about 70% baseline intake and given vehicle intraperitoneally showed no decrease in hepatic PDHa compared with control fed animals. The role of prostaglandins was investigated, since many of the biological effects of TNF are mediated by prostaglandins [2,5]. Administration of the cyclo-oxygenase inhibitor ibuprofen (10 mg/kg intraperitoneally, 1 h before TNF at 25 μ g/kg) did not alter PDHa either alone ($15.9 \pm 0.9\%$, $n = 4$) or in combination with TNF ($17.1 \pm 3.4\%$, $n = 4$). Rats given TNF (25 μ g/kg) daily for 4–7 days (until tolerance to the anorexic

effect had developed) did not exhibit any change in hepatic PDHa in comparison with control values (Table 1).

Effects of combined TNF plus IL-1 on PDHa

TNF and IL-1 often exhibit synergism in their actions [1,34]. The combination of rmIL-1 α (6 μ g/kg) with rhTNF α (25 μ g/kg) after a 20 h fast did not change hepatic PDHa from control values (Table 1). IL-1 α given alone at 6 μ g/kg had no effect on PDHa. Higher doses of TNF (250 μ g/kg) combined with IL-1 α (6 μ g/kg) still did not alter PDHa, despite a marked decrease in food intake to approx. 20–40% of control values. To test whether decreased energy intake was obscuring monokine-induced activation of PDH, we administered glucose intravenously at 25–30 mg/min per kg for 2 h in lieu of oral refeeding to 20 h-fasted catheterized rats, beginning 1 h after administering TNF (25 μ g/kg) plus IL-1 (6 μ g/kg). Hepatic PDHa was not altered by treatment with TNF plus IL-1 (Table 1) in comparison with intravenously glucose-infused controls. Other rats were pair-fed orally to 20–40% of control intake, to match the acute food-intake-decreasing effect of combined TNF/IL-1 treatment. These animals did not exhibit significantly different PDHa in comparison with the rats treated with TNF plus IL-1. Finally, in chronically meal-fed rats fasted for 45 h and then refed with chow for 2 h, administration of TNF (25 μ g/kg) plus IL-1 (6 μ g/kg) had no significant effect on PDHa compared with vehicle administration. Fasting for 45 h before refeeding did result in lower hepatic PDHa compared with preceding 20 h fasts (Table 1), consistent with our previous findings [26].

Table 1. Effects of cytokines on PDHa and food intake in chronically meal-fed rats

Rats were maintained on a chronic meal-feeding regimen consisting of a daily 3 h meal of ground chow. Cytokines or vehicle were administered intraperitoneally after 20 h of fasting, and animals were fed 1 h later. Ibuprofen pre-treatment was intraperitoneal (in 0.9% NaCl) given 1 h before TNF or vehicle. Intravenous (i.v.) glucose refeeding was in chronically catheterized animals at a rate of 25 mg of glucose/min per kg for 2 h, begun at the time of the usual daily meal. All animals were killed 2 h after the start of their refeeding period (3 h after administration of cytokines or vehicle). Values shown represent means $\pm$ S.E.M.: * $P < 0.05$ versus control meal-fed group. The absolute value for food intake in the control animals was 16.2 ± 0.5 g/day.

Treatment (n)	PDHa (%)	Food intake (% of baseline)
Meal-fed controls (7)	13.8 ± 2.1	105.4 ± 0.8
TNF (25 μ g/kg) (8)	9.7 ± 1.3	$89.8 \pm 5.4^*$
TNF (50 μ g/kg) (7)	9.8 ± 1.3	$86.9 \pm 7.5^*$
TNF (100 μ g/kg) (7)	10.6 ± 0.8	$78.1 \pm 5.3^*$
TNF (250 μ g/kg) (3)	13.1 ± 2.5	$78.4 \pm 13.0^*$
Pair-fed controls (7)	13.8 ± 3.6	$66 \pm 5.0^*$
Daily TNF (25 μ g/kg) for 4 days (4)	11.6 ± 2.0	97.5 ± 2.2
IL-1 (6 μ g/kg) (5)	12.6 ± 1.1	$42.2 \pm 3.3^*$
TNF (25 μ g/kg) + IL-1 (6 μ g/kg) (8)	11.0 ± 0.2	$48.4 \pm 14.1^*$
TNF (250 μ g/kg) + IL-1 (6 μ g/kg) (4)	11.0 ± 0.3	$25.3 \pm 11.6^*$
Pair-fed controls (7)	13.3 ± 2.2	$31 \pm 6.0^*$
i.v. glucose for 2 h (12)	15.8 ± 1.3	—
TNF (25 μ g/kg) + IL-1 (6 μ g/kg) + i.v. glucose for 2 h (6)	16.3 ± 2.7	—
45 h fasted (8)	$7.45 \pm 0.47^*$	—
45 h fasted + refed 2 h (4)	11.4 ± 1.5	—
45 h fasted + TNF (25 μ g/kg) + IL-1 (6 μ g/kg) + refed 2 h (4)	10.1 ± 1.4	—

Table 2. Effects of combined TNF (25 µg/kg) plus IL-1 (6 µg/kg) in rats fed *ad libitum*

Values represent mean ± S.E.M., for the numbers of animals in parentheses. Rats fed *ad libitum* were given cytokines or vehicle intraperitoneally, then killed 3 h later. Measurements of lipogenesis *de novo* were performed in chronically catheterized animals as described in the text, by administering [2-¹³C]acetate with m.s. analyses. Fractional contribution (%) from lipogenesis *de novo* represents the percentage of VLDL-palmitate derived from the 'de novo' pathway. Circulating TG derived from lipogenesis *de novo* is calculated as the fractional contribution multiplied by plasma TG concentration. Abbreviations: [TG], concn. of TG in plasma; $-k$, rate constant of ATP-dependant inactivation of PDH (min^{-1}). * $P < 0.05$ by unpaired t test.

Group	PDHa (%)	PDH kinase ($-k$)	Plasma [TG] (mg/dl)	Fractional contribution from lipogenesis <i>de novo</i> (%)	Circulating TG derived from lipogenesis <i>de novo</i> (mg/dl)	Liver glycogen (mg/g wet wt.)	Plasma glucose (mg/dl)	Portal insulin (μ -units/ml)
Controls	14.2 ± 0.8 (9)	0.831 ± 0.205 (3)	109 ± 9 (11)	2.39 ± 2.11 (3)	2.6 ± 2.3 (3)	41.9 ± 9.9 (7)	104 ± 23 (8)	140 ± 20 (8)
+IL-1/TNF	17.2 ± 1.1* (13)	0.772 ± 0.097 (4)	172 ± 28* (7)	8.68 ± 1.8 (7)	14.9 ± 3.0* (7)	19.2 ± 3.8* (15)	155 ± 71 (11)	340 ± 43* (20)
<i>P</i>	0.05	0.78	0.02	0.07	0.03	0.02	0.07	0.01

Effects of TNF and IL-1 on PDHa in rats fed *ad libitum*

These results show that the monokines TNF and IL-1 when given before refeeding have no stimulatory effect on hepatic PDHa after a preceding 20 h or 45 h fast in chronically meal-fed rats. In contrast, intraperitoneal administration of rhTNF α (25 µg/kg) plus rmIL-1 α (6 µg/kg) to rats fed *ad libitum* increased PDH significantly ($P < 0.05$), by approx. 21% (Table 2).

Effects of TNF and IL-1 on PDHa in rats fed sucrose *ad libitum*

Feingold *et al.* [35] recently reported that rats fed on 76% sucrose diet for 3 days exhibited a greater stimulation of lipogenesis in response to TNF than did chow-fed rats. We measured the response of hepatic PDHa to TNF (25 µg/kg) plus IL-1 (6 µg/kg) in rats fed on 76% sucrose *ad libitum* for 3 days. The PDH value in these sucrose-fed rats given vehicle injections was $15.4 \pm 1.5\%$ (mean ± S.E.M., $n = 6$), and that in monokine-treated sucrose-fed rats was $15.8 \pm 1.2\%$ ($n = 6$). Group differences were not significant. Thus hepatic PDHa did not increase after TNF/IL-1 administration in sucrose-fed rats, despite the reported exaggeration of cytokine-induced stimulation of lipogenesis *de novo* by this dietary regimen [35].

Plasma TG response to monokines in rats fed *ad libitum* compared with meal-fed rats

The plasma TG response to recombinant monokines in different groups also depended on prior feeding state. TNF with or without IL-1 treatment did not increase plasma TG concentrations in previously fasted rats compared with controls. Administration of TNF at 25, 100 or 250 µg/kg 1 h before the daily meal did not change plasma TG [20 ± 7 ($n = 4$), 39 ± 4 ($n = 8$) and 36 ± 7 ($n = 3$) respectively; means ± S.E.M.] compared with vehicle-injected controls [32 ± 6 mg/dl ($n = 7$)]. IL-1 (6 µg/kg) also had no effect [24 ± 4 mg/dl ($n = 5$)]. Combination of high dose of TNF (250 µg/kg) plus IL-1 (6 µg/kg) with intravenous glucose feeding at 25 mg/min per kg failed to increase TG concentrations [38 ± 4 mg/dl ($n = 6$)]. In contrast, rats fed *ad libitum* administered combined TNF (25 µg/kg) plus IL-1 (6 µg/kg) exhibited 59% higher TG concentrations than vehicle-injected controls ($P < 0.05$, Table 2).

Hepatic lipogenesis *de novo* in response to IL-1 and TNF

The contribution from hepatic lipogenesis *de novo* to VLDL-fatty acid was measured with radioisotopes ([2-¹⁴C]acetate) or stable isotopes ([2-¹³C]acetate) by using the xenobiotic probe

Table 3. Influence of prior nutritional state on TNF stimulation of lipogenesis *de novo* from [2-¹⁴C]acetate

Lipogenesis *de novo* was measured from [2-¹⁴C]acetate incorporation into VLDL-fatty acids in catheterized rats, as described previously [24] and in the text. The 20 h-fasted animals were chronically meal-fed and were given TNF or vehicle 1 h before the end of their usual interprandial period (21 h), but feeding was then withheld or was with intravenous glucose (25 mg/min per kg for 2 h) before killing, in order to avoid potential confounding effects of TNF on food intake. Rats fed *ad libitum* were given TNF or vehicle, and then food was removed for 3 h. All rats were killed 3 h after injections. Fractional contribution from lipogenesis *de novo* (%) represents the percentage of VLDL-palmitate derived from the *de novo* pathway. Circulating TG derived from lipogenesis *de novo* (%) is calculated as the fraction of VLDL-TG fatty acids derived from lipogenesis *de novo* multiplied by TG concentration (see the text). Values represent means ± S.E.M.: * $P < 0.05$ versus controls fed *ad libitum*.

Treatment group (<i>n</i>)	Fractional contribution from lipogenesis <i>de novo</i> (%)	Circulating TG derived from lipogenesis <i>de novo</i> (mg/dl)
20 h-fasted controls (13)	1.20 ± 0.22	0.36 ± 0.10
20 h-fasted + TNF (50 µg/kg) (5)	0.94 ± 0.13	—
20 h-fasted/refed intravenous glucose (10)	0.55 ± 0.16	0.19 ± 0.12
20 h-fasted/refed intravenous glucose + TNF (50 µg/kg) (3)	0.83 ± 0.11	0.32 ± 0.09
Controls fed <i>ad libitum</i> (3)	1.43 ± 0.66	1.54 ± 0.70
Fed <i>ad libitum</i> + TNF (50 µg/kg) (4)	4.13 ± 1.34*	7.10 ± 2.38*

technique for sampling hepatic acetyl-CoA [24,25]. The fractional contribution from lipogenesis *de novo*, measured with radioisotopes, was low in 21 h-fasted rats and was not increased by administration of TNF (50 µg/kg) (Table 3). Infusion of glucose intravenously at 25 mg/min per kg for 5 h starting 1 h after administration of recombinant monokines did not increase lipogenesis. In rats fed *ad libitum*, however, administration of TNF significantly stimulated fractional lipogenesis *de novo* ($P < 0.05$). When expressed as the concentration of VLDL-TG attributable to lipogenesis *de novo* (calculated as the contribution from lipogenesis *de novo* to TG multiplied by the plasma TG concentration), the differential effects of IL-1 plus TNF in previously fasted rats compared with rats fed *ad libitum* were more pronounced (no significant TNF effect in previously fasted

Fig. 1. Relationship between fractional hepatic lipogenesis *de novo* (% of VLDL-palmitate derived from the pathway of lipogenesis *de novo*) and hepatic PDHa (% of active form) in rats infused with [$2\text{-}^{13}\text{C}$]acetate

Measurements of PDHa and lipogenesis *de novo* are described in the text. Correlation coefficient is shown.

intravenously glucose-fed rats, compared with a 4.6-fold increase in rats fed *ad libitum*; $P < 0.05$). The results obtained with stable isotopes were similar (Table 2). In rats fed *ad libitum*, IL-1 (6 $\mu\text{g/kg}$) combined with TNF (25 $\mu\text{g/kg}$) increased 3.6-fold the fractional contribution from lipogenesis *de novo* to VLDL-palmitate ($P = 0.07$, Table 2). When expressed as concentration of TG derived from lipogenesis *de novo*, TNF plus IL-1 had a 5.7-fold stimulatory effect in rats fed *ad libitum* ($P = 0.03$, Table 2), whereas in 20 h fasted rats given glucose intravenously starting 1 h after administration of monokines, treatment with the recombinant monokines had no effect. Accordingly, the lipogenesis results were similar to the results for plasma TG and, with regard to group means, the results for PDHa. There was essentially no correlation between hepatic PDHa and lipogenesis *de novo* in individual animals, however (Fig. 1; $r^2 = 0.05$, not significant).

Effects of monokines on PDH kinase in rats fed *ad libitum*

PDH kinase activity was measured in isolated hepatic mitochondria from control and monokine-treated rats fed *ad libitum* (Table 2), since this group showed an effect of monokines on PDHa. Kinase activity is expressed as the rate constant ($-k$) for ATP-dependent inactivation of PDH activity in mitochondrial extracts [29]. There was no significant difference between PDH kinase activities in control and monokine-treated animals fed *ad libitum* ($P = 0.78$). Accordingly, the increase in PDHa induced by treatment with IL-1 plus TNF in rats fed *ad libitum* cannot be explained by decreased activity of its inhibitory regulator, PDH kinase.

Liver glycogen content, blood glucose and insulin concentrations

Liver glycogen content in meal-fed rats after their 21 h interprandial fast was less than 5 mg/g wet wt. Intravenous refeeding of 21 h-fasted rats with glucose for 2 h at 25 mg/min per kg increased glycogen content to 9.8 ± 2.9 mg/g wet wt. ($n = 11$), and this was not significantly altered by pre-treatment with TNF plus IL-1 1 h before intravenous glucose refeeding (7.0 ± 2.2 mg/g wet wt., $n = 10$). In contrast, in control rats fed *ad libitum*, the liver glycogen content was 2-fold higher than in rats fed *ad libitum* treated with TNF (25 $\mu\text{g/kg}$) plus IL-1 (6 $\mu\text{g/kg}$) ($P < 0.05$, Table 2). Plasma glucose concentrations were also higher, but these differences did not reach significance ($P = 0.07$, Table 2), whereas portal insulin concentrations were

significantly higher ($P < 0.01$, Table 2), in the monokine-treated group.

DISCUSSION

Our results demonstrate that recombinant monokines influence hepatic PDHa in the rat modestly at best. A complex relationship between type of experimental inflammation and liver or muscle PDHa has been reported previously [3,36]. We report here that TNF does not have a stimulatory effect on hepatic PDHa or lipogenesis *de novo* in rats depleted of carbohydrate reserves by a preceding fast, but does have a significant stimulatory effect in rats fed *ad libitum*. The dependence of recombinant cytokine effects on prior nutritional state is consistent with previous reports measuring lipogenesis [35,37]. It was recently reported [37] that a 24 h fast prevented the monokine-induced stimulation of $^3\text{H}_2\text{O}$ incorporation into lipids in rats previously fed *ad libitum*. Our lipogenesis results confirm this observation (Tables 2 and 3) and reveal a similar pattern for PDHa and serum TG (Tables 1 and 2). Of interest, nutrient status at the time of monokine administration appears to be the key permissive variable, since intravenous glucose administration begun in overnight-fasted rats 1 h after monokine administration caused no increase in PDHa, serum TG or lipogenesis *de novo* (Tables 1 and 3). Stimulation of hepatic glycogenolysis by monokines (demonstrated for IL-6 [38]) might stimulate flux through PDH and synthesis of new fat by non-covalent substrate-level effects. Another metabolic consequence of glycogenolysis might be to increase plasma glucose and insulin concentrations (Table 2), thus decreasing adipocyte lipolysis and fatty acid delivery to the liver, with secondary activation of PDH [22]. It is not clear to us how prior nutritional state would exert a permissive effect, although continuing glucose infusion would not, if stimulation of glycogenolysis is the operative mechanism. One possibility would be if the glycogenolytic effect were less potent during refeeding than before it, as might occur if there were synergism between glucagon and cytokines, which has been reported for hepatic amino acid uptake [39]. Other explanations might include an ability of insulin to overcome the glycogenolytic effect, or pharmacokinetic explanations (e.g., if the cytokine stimulatory effect were potent but short-lived and had worn off by the time that most glycogen was being deposited during refeeding). These remain speculations, but are experimentally testable. In any event, since hepatic glycogen stores in normal humans last longer than in rodents, remaining at roughly half of fed levels after 24 h of fasting [40], monokines might still have a stimulatory effect on PDHa or lipogenesis in briefly anorexic or chronically semi-starved humans, if a similar dependence on carbohydrate stores also applies in man. Other potential mechanisms by which monokines might stimulate lipogenesis (e.g. increased substrate provision from muscle proteolysis) are not addressed by our study. The results of the present study suggest that monokines do not directly increase hepatic PDHa and thereby increase flux of carbohydrates into fats; rather, any effects appear to be indirect and dependent on provision of substrates. In contrast, skeletal-muscle PDHa decreases while PDH kinase activity increases, in response to experimental sepsis or sterile inflammation in rats fed *ad libitum* [36]. Differences between tissues, diet and experimental models must all be taken into account when studying PDH in this context.

Hypertriglyceridaemia induced by monokine administration and fructose-induced hypertriglyceridaemia [27] share the stimulation of lipogenesis *de novo* and presumably pyruvate flux through hepatic PDH, but apparently differ with regard to the role of PDH phosphorylation state. The monokine-induced increase in PDHa was only to 121 % of control values in rats fed

ad libitum, whereas the increase in lipogenesis *de novo* was to 350–550 % of control values; moreover, there was no significant change in PDH kinase activity in these animals and no change in PDHa in sucrose-fed animals. In comparison, dietary fructose increased PDHa to 144 % of control values while increasing lipogenesis *de novo* proportionately (to 168 %) and decreasing PDH kinase activity by two-thirds [27]. A reasonable inference would be that any increase in flux through hepatic PDH induced by monokines is not predominantly due to covalent modification of PDH (dephosphorylation). This notion is supported by comparison of hepatic lipogenesis to PDHa within individual animals infused with [^{13}C]acetate (Fig. 1). Some of the highest lipogenesis values were associated with relatively low values of PDHa, and the converse also held. The inability of TNF to increase hepatic PDHa in sucrose-fed rats, despite reportedly greater stimulation of lipogenesis under these dietary conditions [35], also suggests a substrate-mediated (non-covalent) mechanism for increasing flux through PDH.

The quantitative effect of IL-1 plus TNF on hepatic lipogenesis in rats fed *ad libitum* can be estimated by combining our data with literature values for $^3\text{H}_2\text{O}$ incorporation [41,42] and VLDL production rates [43,44]. If approx. 2 % of circulating TG-fatty acids is derived from the pathway of lipogenesis *de novo* in rats fed *ad libitum* (Table 2, and [25]), and VLDL-TG turnover is approx. 2–4 mg/min per kg [43,44], roughly 0.04–0.08 mg of TG-fatty acid/min per kg or 15–20 mg of fatty acid/day must be secreted by liver under normal conditions of feeding *ad libitum*. This compares rather well with published $^3\text{H}_2\text{O}$ incorporation rates of 3–6 $\mu\text{mol } ^3\text{H}_2\text{O}/\text{h}$ per g of liver [41,42], equivalent to 0.037–0.073 mg of fatty acid/min per kg body wt., by using an H/C incorporation ratio of 0.87 or 14 $\mu\text{mol}/\text{mol}$ of palmitate [41] and an average fatty acid length of C_{16} [42]. Monokine treatment increased the fractional value to approx. 8.5 % of circulating TG-fatty acids (Table 2) in rats fed *ad libitum*, which would represent 0.16–0.32 mg of TG-fatty acid/min per kg even without taking into account the roughly 2-fold stimulation of total VLDL-TG production that has been reported to result from monokine treatment [11,12]. Synthesis of new fat by the liver would therefore consume a minimum of 0.45–0.90 mg of glucose/min per kg. Hence this pathway could represent a significant drain on scarce carbohydrate stores and on gluconeogenic precursors in times of food deprivation. In contrast, under normal well-fed conditions increased synthesis *de novo* would have little impact on glucose carbon reserves [24,25,45]. It has recently been suggested that increased lipogenesis owing to infection or monokines may have a protective function in the host defence against endotoxaemia [46]; even so, the protein-catabolic effects of this pathway would be minimized by a dependence on existing glycogen reserves. Conversion of partially depleted carbohydrate stores into fat would also seem maladaptive in the setting of chronic wasting conditions, however, so this action of monokines might still have deleterious consequences for preservation of lean tissue, particularly if monokines concurrently suppress appetite (Table 1; [32]). The physiological and pathophysiological consequences of monokine-induced changes in hepatic PDH flux require further study.

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