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Circulating Tumor Necrosis Factor-α Levels and Lipid Abnormalities in Patients with Cystic Fibrosis

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ABSTRACT. Hyperlipidemia is prominent among the disturbances in intermediary metabolism that occur subsequent to infections by microorganisms. The response to such infections is known to involve several cell types and is mediated by cytokines. We hypothesized that metabolic lipid disturbances seen during infection in cystic fibrosis (CF) patients may partly be the result of excessive tumor necrosis factor-α (TNF-α), a proinflammatory cytokine known to cause a large spectrum of pathophysiologic alterations, including impaired lipid metabolism. Therefore, we determined the circulating concentration of TNF-α and analyzed its relationship to lipid and lipoprotein levels, as well as lipoprotein lipase activity, in 31 CF patients. Plasma TNF-α values were significantly ($p < 0.01$) elevated in patients with CF compared with controls. The CF subjects were found to have decreased plasma cholesterol (25%), LDL cholesterol (35%), and HDL cholesterol (19%) concentrations, whereas plasma triglycerides were significantly increased ($p < 0.001$). The apo A-I level was reduced ($p < 0.005$), whereas apo B levels were normal. Low levels of the major essential fatty acids were found in the plasma of the CF patients, and the triene/tetraene ratio confirmed their essential fatty acid deficiency. Postheparin lipolytic activity was lower in CF patients than in controls, and the decreased activity was accounted for primarily by a decline in hepatic lipase. A significant positive correlation ($p < 0.001$, $r = 0.70$) was found between TNF-α and plasma triglyceride levels. However, no association was noted between TNF-α and essential fatty acid, cholesterol, or lipoprotein cholesterol levels, or with lipoprotein lipase activity. These studies suggest that TNF-α contributes to the hypertriglyceridemia in CF patients primarily by stimulating hepatic lipogenesis, rather than inhibiting triglyceride clearance. (*Pediatr Res* 34: 162–166, 1993)

Abbreviations

CF, cystic fibrosis
EFA, essential fatty acid

CF is characterized by exocrine dysfunction, abnormal sweat electrolytes, and the secretion of viscous mucus (1). These disturbances result in repeated airway infections and obstruction, pancreatic insufficiency, and cirrhosis (1, 2). Advances in pancreatic enzyme replacement therapy have rendered pancreatic failure much less of a problem. Nevertheless, chronic respiratory disease, present in 95% of CF patients, remains the major cause of morbidity and mortality (1, 2). The secretion of dehydrated mucus impairs airway mucociliary clearance and predisposes patients to recurrent bronchial infections (3, 4). The CF airway shows a particular propensity to colonization and recurrent infection with the bacterium *Pseudomonas aeruginosa* progressively destroying the lung, leading to respiratory failure (5, 6). Chronic bacterial infections have been implicated in disturbances of intermediary metabolism, including hyperlipidemia. The chronic inflammatory process is accompanied by increased systemic concentrations of inflammatory cytokines with diverse biologic effects. TNF-α, also termed cachectin, is a proinflammatory cytokine that has been implicated as an important mediator in several chronic inflammatory disorders (7, 8). In addition to its central role in inflammation and immunity, TNF's numerous biologic effects include hyperlipidemia (9, 10). TNF-α causes an increase in triglycerides carried by VLDL as a result of the well-established inhibitory effects of TNF-α on lipoprotein lipase and hepatic lipogenesis (11-13).

We have recently shown that CF patients have abnormalities in lipoprotein concentration, composition, size, and metabolism (14, 15). In addition to EFA deficiency, increased circulating levels of TNF in CF may provide an explanation for the lipid and lipoprotein derangement in CF. To verify this hypothesis, we determined the plasma levels of TNF-α in CF patients and evaluated the correlation between TNF-α and lipid and lipoprotein levels and their metabolism.

PATIENTS AND METHODS

Subjects. We randomly selected 31 young adolescent patients followed at the CF clinic of St.-Justine Hospital (age range 9 to 14 y; 18 males and 13 females). The diagnosis was based on the sweat chloride test and had been previously confirmed by family history of CF, clinical findings of typical pulmonary disease, and/or gastrointestinal disorders. Informed consent was obtained from the parents, and the project was approved by the Ethics Committee of St.-Justine Hospital. The group of CF patients was attending a CF outpatient clinic at the time of sampling. They were consuming a high-energy diet (150% of recommended daily allowance for age), composed of 45 to 50% carbohydrate, 15 to 20% protein, and 35 to 40% lipid. The intake of fat included a 10 to 15% EFA supplement (sunflower or corn oil). All the CF patients were maintained on multivitamin supplements, exogenous pancreatic enzymes, and oral antibiotics. Patient compliance with the dietary intake was evaluated closely by the team of nutritionists during many follow-up visits. When compared

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with controls (C), no abnormalities were noted in their percentage of ideal height 97.9 ± 0.8 (C: 95–105) and percentage of ideal weight for height 95.5 ± 1.7 (C: 95–105). None of these patients had CF-related liver disease, as evidenced by normal liver function tests, including serum bilirubin, 5.2 ± 0.9 μmol/L (C: <15); alanine aminotransferase, 30.3 ± 4.3 μmol/L (<35); and γ-glutamine transferase, 23.7 ± 4.6 μmol/L (<45). The CF patient group had pancreatic insufficiency, as shown by steatorrhea (13.8 ± 1.6 g/24 h), absent in all controls. Their chronic respiratory disease was stable with no evidence of pulmonary exacerbation at the time of study (Schwachman score 77.4 ± 1.5). The CF subjects included EFA-sufficient and EFA-deficient patients. Healthy, age-matched controls ($n = 39$) were recruited for comparative study. They consisted of a group of normal children undergoing dyslipidemia screening. Controls with abnormal plasma lipids or lipoprotein levels were excluded.

Lipid and lipoprotein analyses. Blood samples were collected in EDTA (1 mg/mL) after a 12-h overnight fast. Plasma was separated immediately by low-speed centrifugation (1500 × *g*) at 4°C. The concentrations of total cholesterol and triglycerides were determined enzymatically using a commercial kit (Boehringer Mannheim, Montreal, Canada) as reported previously (14, 16). HDL cholesterol was quantified after the precipitation of VLDL and LDL with phosphotungstic acid (14, 16). LDL cholesterol was measured by the method of Friedewald *et al.* (17). Quantitative measurements of apolipoprotein levels in whole plasma were carried out by nephelometry using a commercial standard supplied by Hoerscht-Behring (Quebec, Canada) (14). The plasma fatty acid composition was analyzed using methods we have described previously (15).

Lipolytic activity measurement. For assay of lipolytic activities, heparin was injected i.v. (10 U/kg body weight), and blood was collected 10 min later into EDTA, as per our established method (14, 16). Total lipolytic activity was measured in plasma with an emulsion of tri[1-^{14}C]oleoylglycerol as substrate (18). Hepatic lipase was quantified in the presence of protamine sulfate, and extralipoprotein lipase activity was calculated as the difference between total lipase activity and hepatic lipase. The extraction of FFA was performed by the procedure of Belfrage and Vaughan (19) as previously described (20).

Quantification of TNF-α. Plasma TNF-α concentrations were assayed by standard RIA kit (InterMed, Markham, Ontario, Canada) and expressed as pg/mL.

Statistical analysis. All values were assessed in duplicate and are expressed as the mean ± SEM. Statistical differences between means were assessed using the two-tailed *t* test. Linear regression analysis was used to assess the correlation between values expressed in graphs as continuous functions.

RESULTS

The patterns of plasma lipids, lipoproteins, and apoproteins for the CF patients and control groups are shown in Table 1. Mean plasma triglyceride levels were elevated in CF patients ($p < 0.001$) compared with controls. Furthermore, significant hypocholesterolemia characterized the CF group, and this was associated with a significant ($p < 0.001$) decrease in both LDL cholesterol and HDL cholesterol fractions. The apo A-I concentration was reduced in CF patients ($p < 0.005$), whereas apo B levels were similar to those in controls.

The plasma fatty acid pattern was also profoundly affected in CF patients (Table 2). Striking changes were observed in the major polyunsaturated fatty acids. Linoleic acid and arachidonic acid were decreased by 27.6 and 22.2%, respectively, compared with control values ($p < 0.001$). On the other hand, palmitoleic acid and eicosatrienoic acid were increased in CF patients by 2.2- and 3.6-fold, respectively ($p < 0.001$). These marked alterations in fatty acids led to a 7-fold ($p < 0.005$) elevation of the 20:3ω9/20:4ω6 ratio, a well-established index confirming EFA deficiency. Similarly, the 16:1ω7/18:2ω6 ratio, which has also been shown to be a good discriminant for EFA deficiency, was four times higher in CF group than in controls ($p < 0.001$).

To assess the potential role of LPL activity in the abnormally elevated plasma triglyceride concentration, lipolytic activity was measured in plasma of CF and control patients (Fig. 1). The hypertriglyceridemia, described above, was associated with a 31% decrease of total postheparin lipolytic activity, compared with controls (9.40 ± 1.02 *versus* 13.57 ± 0.73 μmol FFA/mL/h, $p < 0.005$). Hepatic lipase activity was particularly affected in CF patients (4.59 ± 0.49 *versus* 8.61 ± 0.63, $p < 0.001$).

Plasma TNF-α concentrations (Fig. 2), determined by RIA, were significantly ($p < 0.01$) increased (~3-fold) in CF patients compared with controls (82.7 ± 8.4 *versus* 21.1 ± 7.2 pg/mL). The relationship between plasma TNF-α and plasma triglycerides is presented in Figure 3. A significant correlation was found between plasma triglycerides of CF patients and TNF-α ($r = 0.70$, $p < 0.001$), whereas no correlation was detectable between TNF-α levels and 18:2ω6/20:4ω6, 16:1ω7/18:2ω6, 20:3ω9/20:4ω6, total postheparin lipolytic activity or extrahepatic lipoprotein lipase activity (Table 3). However, a negative correlation was noted between TNF-α and total cholesterol ($r = 0.53$, $p < 0.0025$) as well as hepatic lipase activity ($r = 0.64$, $p < 0.0068$). These correlation coefficients were more elevated ($r > 0.70$) when control values were included in the analysis. Furthermore, exclusion of the patients with an outlying TNF-α levels above 150 pg/mL did not remove the significance of the correlation between TNF-α and plasma triglycerides ($r = 0.52$, $p < 0.005$).

DISCUSSION

The present study demonstrates that pediatric CF patients have markedly abnormal plasma fatty acid patterns, significant alteration in lipid and lipoprotein concentrations, and lipoprotein lipase impairment, in agreement with our previous findings (14, 15). Our results reveal the presence of increased circulating levels of TNF-α, a primary mediator in the pathogenesis of infection, injury, and inflammation. Furthermore, the data obtained suggest an association of elevated TNF-α with hypertriglyceridemia in CF patients.

The hypertriglyceridemia observed in CF patients is remarkable in that these patients often present with malabsorption and malnutrition (21). CF patients are particularly prone to chronic pulmonary infection with *P. aeruginosa*, the most prominant bacterial colonizer (4–6). One cytokine that is being increasingly recognized as a central mediator in a wide spectrum of physiologic and immune functions is macrophage-derived TNF-α (22). The production or administration of high concentrations of TNF-α can cause a large spectrum of metabolic and pathophysiologic alterations, including acidosis (23), elaboration of stress hormones (24), a decrease in the transmembrane potential

Table 1. *Plasma lipids, lipoproteins, and apoproteins**

Subjects	Triglycerides (mmol/L)	Total cholesterol (mmol/L)	LDL cholesterol (mmol/L)	HDL cholesterol (mmol/L)	Apo A-I (g/L)	Apo B (g/L)
CF	1.29 ± 0.13	2.93 ± 0.18	1.61 ± 0.12	0.97 ± 0.06	1.15 ± 0.05	0.74 ± 0.05
Controls	0.64 ± 0.03	3.91 ± 0.10	2.48 ± 0.10	1.20 ± 0.05	1.32 ± 0.03	0.72 ± 0.03
p	0.001	0.001	0.001	0.005	0.005	NS

* Values are mean ± SEM.

Table 2. *Plasma fatty acids and indices of EFA deficiency**

Subjects	Fatty acids: 16:1ω7 (mol %)	18:2ω6 (mol %)	20:4ω6 (mol %)	20:3ω9 (mol %)	EFAD ratios: 16:1ω7 / 18:2ω6	20:3ω9 / 20:4ω6
CF (n = 31)	3.59 ± 0.26	21.30 ± 0.99	6.15 ± 0.30	0.40 ± 0.07	0.232 ± 0.043	0.098 ± 0.026
Controls (n = 39)	1.62 ± 0.08	29.44 ± 0.49	7.91 ± 0.19	0.11 ± 0.005	0.057 ± 0.003	0.014 ± 0.0006
p	0.001	0.001	0.001	0.001	0.001	0.005

* Results are expressed as mol % of total fatty acids. All data represent mean values ± SEM.

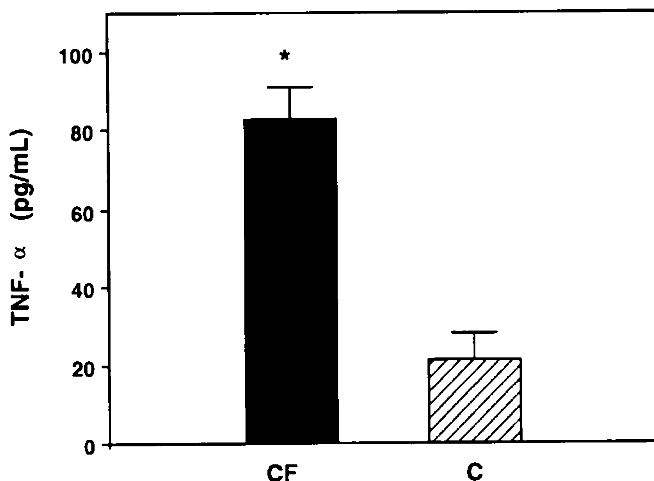

Fig. 1. Plasma TNF-α in CF and control (C) subjects; *, $p < 0.01$. The multiplication factor is 5.7×10^{-5} to convert TNF-α values to pmol/L.

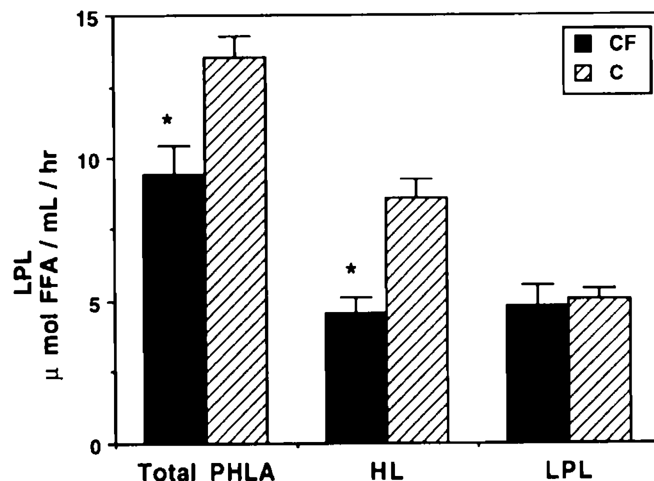

Fig. 2. Postheparin lipoprotein lipase activity in CF and control (C) subjects. *PHLA*, postheparin lipolytic activity; *LPL*, extrahepatic lipoprotein lipase activity; *HL*, hepatic lipase activity. *, $p < 0.005$ at least.

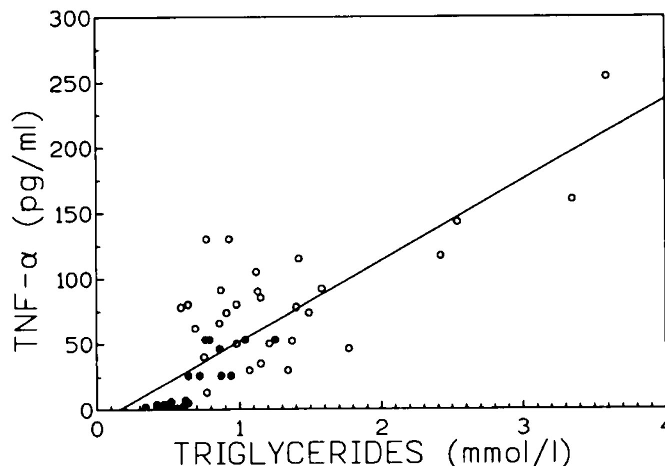

Fig. 3. Correlation between plasma TNF-α and triglyceride levels in CF patients (○) and age-matched healthy controls (●); $r = 0.80$, $p < 0.0001$. The multiplication factor is 5.7×10^{-5} to convert TNF-α values to pmol/L.

Table 3. *Correlation between TNF-α and biochemical parameters**

	18:2ω6	20:4ω6	16:1ω7 / 18:2ω6	20:3ω9 / 20:4ω6	Total cholesterol	Total PHLA	LPL	HL
TNF-α	−0.28	−0.18	−0.06	−0.00	−0.53†	−0.43	−0.17	−0.64‡

(Column headings grouped under "Parameter".)

* PHLA, postheparin lipolytic activity; HL, hepatic lipase activity.
† $p < 0.0025$.
‡ $p < 0.0068$.

of skeletal muscle (25), and a decrease in blood glucose (26). Similarly, the changes in lipid metabolism noted during infection are now known to be produced by cytokines (7–10). Increased hepatic lipid synthesis *in vivo* and decreased levels of lipoprotein lipase in certain adipose tissues *in vitro* and *in vivo* have been induced by several cytokines, including TNF-α. These considerations prompted us to analyze the relationship between circulating TNF-α levels and plasma triglycerides in CF. We found that circulating TNF-α levels were commonly elevated in patients with CF, and that there was a significant correlation between the absolute concentrations of TNF-α and plasma triglycerides in such patients.

TNF-α may induce hypertriglyceridemia by its inhibitory effect on lipoprotein lipase activity, as well as its enhancement of hepatic lipogenesis (11–13). Decreases in lipoprotein lipase could lead to high plasma triglyceride levels by diminishing the clearance of triglyceride-rich lipoproteins. However, our results did not disclose any correlation between total or extrahepatic postheparin lipoprotein lipase activities and triglycerides or TNF-α. Despite the absence of clinical or biochemical evidence of liver dysfunction in the CF group studied, hepatic lipase level was significantly reduced. However, only a negative correlation was found between hepatic lipase and plasma TNF-α.

Our observations are not at variance with recent data, indicating that TNF-α may induce an increase in serum triglyceride levels *in vivo* by stimulating hepatic lipogenesis and VLDL production. However, these studies were unable to demonstrate that TNF-α is an inhibitor of adipose tissue lipoprotein lipase activity and triglyceride clearance (26). Also of interest are the observations of Suter *et al.* (27), showing elevated plasma levels of other cytokines in CF, including IL-1α and IL-1β. Both IL-1 and interferon-α have also been shown to stimulate hepatic lipogenesis (28). Additional studies are obviously needed to further explore the significance and relationship between various cytokines and lipid metabolic abnormalities in CF patients.

The major effects of severe EFA deficiency include growth failure, a scaly dermatitis, impaired development of reproductive systems, gastrointestinal tract dysfunction, and an increased basal

metabolic rate (29, 30). The CF group studied in this report was not different from controls with respect to growth parameters. In addition, EFA deficiency can decrease resistance to bacterial infection, aggravating the chronic respiratory disease associated with accumulation of tenacious, purulent exudate in secondary bronchi (31). Given that linoleate and arachidonate are required for prostaglandin synthesis, their reduced availability in CF might decrease the formation of these biologically active substances with wide-spread physiologic consequences. Chase and Dupont (32) found that CF patients with low linoleic acid levels had increased prostaglandin $F_{2\alpha}$, whereas others have reported decreased prostaglandin $F_{2\alpha}$ (33). The latter prostaglandin appears to participate in the down-regulation of TNF-α expression and secretion. Prostaglandin E_2 is known to inhibit the production of TNF-α by monocytes (34, 35). Because prostaglandin E_2 is an arachidonic acid metabolite, it was logical to examine the relationship between elevated TNF-α levels in CF patients with EFA deficiency. Our data did not, however, reveal a positive correlation between EFA deficiency and TNF-α. Future directions that need to be explored in the area of inflammation in CF include the relationship of EFA deficiency with other cytokines. This is particularly important in view of the available data demonstrating that prostaglandin E_2 affects the activity of macrophages, resulting in reduced IL-1 secretion and potentially impairing host immune response to infection.

The negative correlation found between circulating TNF-α levels and total cholesterol as well as hepatic lipase is intriguing. So far, we have no plausible explanation for these findings. Whether TNF-α reduces the synthesis of cholesterol or stimulates its degradation is not yet known. Harada *et al.* (36) have recently investigated the effects of recombinant human TNF-α on the cellular binding of human LDL to skin fibroblasts. Their results indicated that TNF-α increased LDL receptor activity, which might contribute to the rapid disappearance of peripheral cholesterol.

As mentioned before, CF patients are particularly prone to chronic pulmonary infections, with multiple cytokines produced by immune cells to eliminate invading microorganisms. Recently, it has been suggested that increased plasma triglyceride levels could be beneficial during infections. VLDL particles can protect from the toxicity of endotoxins secreted by microorganisms (37), and furthermore, HDL binds endotoxin (38). Other studies have shown that lipoproteins are able to inactivate viruses (39). These observations suggest that the hyperlipidemia occurring in CF is part of the body's host defense mechanisms.

In summary, our study demonstrated an abnormally elevated concentration of circulating TNF-α in CF patients. Our results suggest that TNF-α may participate in the hypertriglyceridemia of CF patients by stimulating hepatic lipogenesis rather than inhibiting TG clearance by lipoprotein lipase. Despite the detected correlation between TNF-α and hypertriglyceridemia, additional investigation is called for to identify the underlying mechanisms.

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REFERENCES

1. Welsh M, Fick RB 1987 Cystic fibrosis. J Clin Invest 80:1523–1526
2. Boat T, Welsh MJ, Beaudet AL 1989 Cystic fibrosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The Metabolic Basis of Inherited Disease, 6th Ed. McGraw-Hill, New York, pp 2649–2680
3. Doershuk CF, Boat TF 1987 Cystic fibrosis. In: Behrman RE, Vaughan CF III, Nelson WE (eds) Nelson Textbook of Pediatrics, XIIIth Ed. WB Saunders, Philadelphia, pp 926–935
4. Marks MI 1981 The pathogenesis and treatment of pulmonary infections in patients with cystic fibrosis. J Pediatr 88:173–179
5. Suter S, Schaad UB, Tegner H, Ohlsson K, Desgrand-champs D, Waldvogel FA 1986 Levels of free granulocyte elastase in bronchial secretions from patients with cystic fibrosis. Effect of antimicrobial treatment against *Pseudomonas aeruginosa*. J Infect Dis 153:902–909
6. Suter S, Schaad UB, Roux L, Nydgger UE, Waldvogel FA 1984 Granulocyte neutral proteases and *Pseudomonas* elastase as possible causes of airway damage in patients with cystic fibrosis. J Infect Dis 149:523–531
7. Beutler B, Ceranin A 1987 Cachectin: more than a tumor necrosis factor. N Engl J Med 136:379–385
8. Tracey KV, Vlassara H, Cerami A 1989 Cachectin/tumor necrosis factor. Lancet 1:1122–1125
9. Semb H, Peterson J, Tavernier J, Olivecrona T 1987 Multiple effects of tumor necrosis factor on lipoprotein lipase *in vivo*. J Biol Chem 262:8390–8394
10. Feingold KR, Soued M, Adi S, Staprans I, Shigenaga J, Doerrler W, Moser A, Grunfeld C 1990 Tumor necrosis factor-increased hepatic very-low-density lipoprotein production and increased serum triglyceride levels in diabetic rats. Diabetes 39:1569–1574
11. Enerback S, Semb H, Tavernier J, Bjursell G, Olivecrona T 1988 Tissue-specific regulation of guinea pig lipoprotein lipase: effects of nutritional state and of tumor necrosis factor on mRNA levels in adipose tissue, heart and liver. Gene 64:97–106
12. Feingold KR, Grunfeld C 1987 Tumor necrosis factor-alpha stimulates hepatic lipogenesis in the rat *in vivo*. J Clin Invest 80:184–190
13. Grunfeld C, Verdier JA, Neese R, Moser AH, Feingold KR 1988 Mechanisms by which tumor necrosis factor stimulates hepatic fatty acid synthesis. J Lipid Res 29:1327–1335
14. Levy E, Lepage G, Bendayan M, Ronco N, Thibault L, Galeano N, Smith L, Roy CC 1989 Relationship of decreased hepatic lipase activity and lipoprotein abnormalities to essential fatty acid deficiency in cystic fibrosis patients. J Lipid Res 30:1197–1209
15. Lepage G, Levy E, Ronco N, Smith L, Galeano N, Roy CC 1989 Direct transesterification of plasma fatty acids for the diagnosis of essential fatty acid deficiency in cystic fibrosis. J Lipid Res 30:1483–1490
16. Levy E, Thibault LA, Roy CC, Bendayan M, Lepage G, Letarte J 1988 Circulating lipids and lipoproteins in glycogen storage disease type I with nocturnal intragastric feeding. J Lipid Res 29:215–226
17. Friedewald WT, Levy RI, Fredrickson DS 1972 Estimation of the concentration of low-density lipoprotein cholesterol in plasma without the use of the preparative ultracentrifuge. Clin Chem 18:499–504
18. Krauss RM, Levy RI, Frederickson DS 1974 Selective measurement of two lipase activities in post-heparin plasma from normal subjects and patients with hyperlipoproteinemia. J Clin Invest 54:1107–1124
19. Belfrage P, Vaughan M 1969 Simple liquid-liquid partition system for isolation of labeled oleic acid from mixtures with glycerides. J Lipid Res 10:341–344
20. Levy E, Thibault L, Garofalo C, Messier M, Lepage G, Ronco N, Roy CC 1990 Combined (n-3 and n-6) essential fatty acid deficiency is a potent modulator of plasma lipids, lipoprotein composition, and lipolytic enzymes. J Lipid Res 31:2009–2017
21. Roy CC, Weber AM, Lepage G, Smith L, Levy E 1988 Digestive and absorptive phase anomalies associated with the exocrine pancreatic insufficiency of cystic fibrosis. J Pediatr Gastroenterol Nutr 7(suppl 1):S1–S7
22. DeForge LE, Nguyen DT, Kunkel SL, Remick DG 1990 Regulation of the pathophysiology of tumor necrosis factor. J Lab Clin Med 116:429–438
23. Remick DG, Kunkel RG, Larrick JW, Kunkel SL 1987 Acute *in vivo* effects of human recombinant tumor necrosis factor. Lab Invest 56:583–590
24. Tracey KJ, Lowry SF, Tahey TJ 1987 Cachectin/tumor necrosis factor induces lethal shock and stress hormone responses in the dog. Surg Gynecol Obstet 164:415–422
25. Tracey KJ, Lowry SF, Beutler B, Cerami A, Albert JD, Shires GT 1986 Cachectin/tumor necrosis factor mediates changes of skeletal muscle plasma membrane potential. J Exp Med 164:1368–1373
26. Grunfeld C, Palladino Jr MA 1990 Tumor necrosis factor: immunologic, antitumor, metabolic and cardiovascular activities. Adv Intern Med 35: 45–72
27. Suter S, Schaad UB, Roux-Lombard P, Girardin E, Grau G, Dayer JM 1989 Relation between tumor necrosis factor-alpha and granulocyte elastase-α1-proteinase inhibitor complexes in the plasma of patients with cystic fibrosis. Am Rev Respir Dis 140:1640–1644
28. Feingold KR, Soued M, Jerio MK, Moser AH, Dinarello CA, Grunfeld C 1989 Multiple cytokines stimulate hepatic lipid synthesis *in vivo*. Endocrinology 125:267–274
29. Aaes-Jorgensen E 1961 Essential fatty acids. Physiol Rev 41:1–51
30. Levy E, Garofalo C, Thibault L, Dionne S, Daoust L, Lepage G, Roy CC 1992 Intraluminal and intracellular phases of fat absorption are impaired in essential fatty acid deficiency. Am J Physiol 262:G319–G326
31. Elliott RB 1976 Therapeutic trial of fatty acid supplementation in cystic fibrosis. Pediatrics 57:474–479
32. Chase HP, Dupont J 1978 Abnormal levels of prostaglandins and fatty acids in blood of children with cystic fibrosis. Lancet 2:236–238
33. Mischler EH, Parrell SW, Farrell PM, Raynor WJ, Lemen RJ 1986 Correction of linoleic acid deficiency in cystic fibrosis. Pediatr Res 20:36–41
34. Spengler RN, Spengler ML, Lincoln P, Remick DG, Strieter RM, Kunkel SL 1989 Dynamics of dibutyryl cyclic AMP- and prostaglandin E_2-mediated

suppression of lipopolysaccharide-induced tumor necrosis factor alpha gene expression. Infect Immunol 57:2837–2841
35. Spengler RN, Spengler ML, Strieter RM, Remick DG, Larrick JW, Kunkel SL 1989 Modulation of tumor necrosis factor-alpha gene expression: desensitization of prostaglandin E_2-induced suppression. J Immunol 142:4346–4351
36. Harada K, Shimano H, Kawakami M, Ishibashi S, Gotoda T, Mori N, Takaku F, Yamada N 1990 Effect of tumor necrosis factor/cachectin on the activity of the low density lipoprotein receptor on human skin fibroblasts. Biochem Biophys Res Commun 172:1022–1027
37. Harris HW, Grunfeld C, Feingold KR, Rapp JR 1990 Human VLDL and chylomicrons can protect against endotoxin-induced death in mice. J Clin Invest 86:696–702
38. Ulevitch RJ, Johnston AR, Weinstein DB 1979 New function for high density lipoproteins. Their participation in intravascular reactions of bacterial lipopolysaccharides. J Clin Invest 64:1516–1524
39. Sernatinger J, Hoffman A, Hardman D, Kane JP, Levy JA 1988 Neutralization of mouse xenotropic virus by lipoproteins involves binding to the virus. J Gen Virol 69:2651–2661

Announcement

The Australian NHMRC Twin Registry: A Resource for Pediatric Research

The Australian NHMRC Twin Registry contains more than 4000 pairs of twins under 10 years old and 3000 more up to the age of 18 years. The parents of these twins have volunteered to consider requests from researchers for studies in *bona fide* projects approved by the Registry. This represents a major resource for studies in pediatric and adolescent epidemiology. There are numerous ways in which twins can be used to address scientific and medical questions. Some examples include examining genetic and environmental variation, co-twin control studies based on disease discordant or exposure discordant pairs, longitudinal studies, and studies of gene-environment interaction (Clifford and Hopper: The Australian NHMRC Twin Registry. A resource for the Australian scientific community. Med J Aust 60:149, 1986). Although there have been more than 70 projects conducted over the last 12 years on the 17000 adult pairs in the Registry, to date there have been few studies making use of the younger pairs. Baseline information has been computerized on about 50% of these pairs. Applications to use the Registry can be made from researchers throughout the world. There may be some charge, depending on costs and level of work required by local staff. All applications are reviewed by the Executive Committee of the Registry, and advice on study design and practicalities is available. *For more information, contact:* Dr. John L. Hopper, Director, Australian NHMRC Twin Registry, 151 Barry Street, Carlton, Victoria 3053, Australia.