Interleukin-1-induced Anorexia in the Rat

Influence of Prostaglandins

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Abstract

The anorexia associated with acute and chronic inflammatory or infectious conditions is poorly understood. Our objectives were to explore the anorexigenic effects of interleukin-1 (IL-1) in the rat. Recombinant human (rh) IL-1β, murine (rm) IL-1α and to a lesser extent rhIL-1α significantly reduced food intake at $\geq$ 4.0 μg/kg i.p. but not at lower doses, in young (200–250 g) meal-fed rats on chow diets. The anorexic effect appears to be mediated by prostaglandins since pretreatment with ibuprofen completely blocked it, and a fish oil based diet abolished it, in comparison to corn oil or chow diets. Fish oil feeding also decreased basal and IL-1 stimulated prostaglandin E_2 production by tissues in vitro (liver, brain, peritoneal macrophages) and in the whole body. Constant intravenous infusions of lower doses of IL-1 also diminished food intake, though intravenous boluses did not (reflecting rapid renal clearance). Chronic daily administration of IL-1 caused persistent inhibition of food intake for 7–17 d in chow and corn oil fed rats, but had no effect in fish oil fed rats. There was an attenuation of the effect (tachyphylaxis) after 7 d in corn oil and chow fed rats, but slowed weight gain and lower final weights were observed after 17–32 d of daily IL-1. Old (18–20 mo Fisher 344) rats showed less sensitivity to IL-1 induced anorexia. In conclusion, IL-1 is anorexigenic in the rat, but this is influenced by the structural form of IL-1, the route and chronicity of administration, the source of dietary fat, and the age of the animal. The ability of prior fat intake to influence the anorexic response to IL-1 represents a novel nutrient-nutrient interaction with potential therapeutic implications.

Introduction

Both acute and chronic inflammatory conditions as well as infectious diseases are associated with alterations in nutrition and metabolism (1, 2). One of the most important of these is decreased food intake (anorexia), which can progress to cachexia and death by starvation (3, 4). With its high prevalence in human immunodeficiency virus-infected patients (5–7), cachexia is becoming an increasingly important problem, yet it remains poorly understood. The availability of recombinant cytokines (8, 9) allows experimental testing of new hypotheses about the mediators and mechanisms of the anorexia of inflammation.

Tumor necrosis factor/cachectin (TNF)[1] has received attention in this regard (9–12). Administration of a solution containing macrophage secretory products including TNF suppresses food intake in mice (10) but these preparations contain other cytokines such as IL-1β (13) in addition to TNF. Tumor cells secreting TNF can induce cachexia in mice (11) but again the presence of other mediators cannot be excluded. When given in sublethal doses to mice (12, 13), recombinant TNF results in decreased appetite and weight loss. However, the effect appears to be short lived (12) in addition to requiring escalating doses (13).

The effect of IL-1 on food intake in rats is largely unknown (14). McCarthy et al. (15) reported decreased intake of a liquid diet in rats given recombinant murine IL-1α, but the effect was relatively minor, lasting only for the first hour of refeeding. More recently (16), rhIL-1β administered to ad lib. fed mice at 6 μg/kg per d in two divided doses was reported to decrease food intake by 10–15%, a similar quantitative effect as for rhIL-1α but significantly less than recombinant murine IL-1α given at similar doses (40–50% reduction in intake). The effect of IL-1β on food intake is relevant to interpretation of TNF effects, since TNF stimulates the release of IL-1β (16, 17).

Our objectives in these studies were, first, to develop a quantitative, reproducible and physiologically relevant animal model for the anorexia of inflammatory illnesses, using IL-1. Dose-response relationships, potential mediation by eicosanoid pathways, involvement of central or peripheral appetite regulatory mechanisms, effects of normal aging, differences between the two structural forms of IL-1 and persistence of any effects during chronic IL-1 administration were then examined.

Methods

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) were trained to a single daily meal (meal-fed) after reaching 150 g body wt. Meal-feeding has advantages over ad lib. feeding for studies of drug or hormone effects on appetite (18, 19), in that the effects of anorexigens with a short biological duration of action

Portions of this work have appeared previously in abstract form. 1988. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 2:A1198. (Abstr.)

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Received for publication 2 June 1988 and in revised form 7 March 1989.

J. Clin. Invest.

0021-9738/89/07/0228/08 $2.00
Volume 84, July 1989, 228–235

1. *Abbreviations used in this paper:* C.O., corn oil; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; F.O., fish oil; LA, linoleic acid; TNF, tumor necrosis factor.

are not lost over a 24-h period, since all intake occurs over 3 h. Meal-fed animals grow well and eat amounts comparable to ad lib.-fed rats (20), and the metabolic and enzymatic consequences of meal-feeding have been well characterized (21, 22). Studies were performed at 200–250 g body wt. Aged Fisher 344 rats (18–20 mo old, National Institute of Aging) were used in some studies. All animals were housed in individual cages in a temperature controlled room (22°C) with a 12-h dark/light cycle daily. Animals were fed nonpurified diets consisting of ground Purina Rat Chow (Ralston Purina Co., St. Louis, MO), or purified nutritionally adequate diets containing 10% by weight of corn oil (C.O.) (Mazola; Best Foods, Englewood Cliffs, NJ) or 1.2% C.O. plus 8.8% fish oil (F.O.) (MaxEPA, a gift from R. P. Scherer Co., Troy, MI) for 6 wk. The details of diet preparation and precautions to minimize oxidation of oils have been previously published (22). As previously reported (23), C.O. contained 65.6±0.6% linoleic acid (LA) while MaxEPA contained 2.0±1.4% LA, 16.7±1.8% eicosapentaenoic acid (EPA), and 12.2±1.1% docosahexanoic acid (DHA). The F.O. diet, therefore, provided about 1% by weight of LA. Food was provided daily in spill-proof glass containers placed inside cages. Water was provided ad lib.

RhIL-1β was produced, purified and characterized as described (8) and consisted of the carboxy-terminal 157 amino acids. This and subsequent lots were active in the rabbit pyrogen test at 100 mg/kg and the mouse lymphocyte activating factor (LAF) assay. The endotoxin concentration in the present preparation was < 60 pg/mg. The specific activity as measured by half-maximal units (LAF) on C_3H/He_5 mouse thymocytes was 5.5×10^7 U/mg. The rhIL-1β was diluted in 0.1% serum which had been dialyzed overnight at 4°C then heated to 56°C for 20 min. The diluted rhIL-1β was administered either intravenously or intraperitoneally. RhIL-1α and recombinant murine (rm) IL-1α were generously provided by Dr. Peter Lomedico (Hoffmann-La Roche, Nutley, NJ). The activity of rmIL-1α is 1.3×10^8 U/mg (D10.G4.1 cells, where 1 LAF unit = 20–50 D10 units) and the endotoxin concentration is 0.55 ng/mg protein (LAL). The activity of rhIL-1α is 2.5×10^9 U/mg (D10 assay) and the endotoxin concentration is 65 pg/mg protein (LAL). Meal-fed rats (Chow, C.O., or F.O.) given 0.1% serum i.p. or heat inactivated rhIL-1β i.p. 1 h premeal had no change in food intake from baseline.

The technique for placement and maintenance of indwelling intrajugular silastic catheters has been described elsewhere (25). Catheterization may depress food intake for 24–48 h, therefore no studies were performed for at least 72–96 h after catheter placement, until food intake had stabilized at precatheterization values. A parenteral preparation of ibuprofen (50 mg/ml) was the gift of Upjohn Co. (Kalamazoo, MI) and was administered intravenously or intraperitoneally in sterile 0.45% saline.

48-h urine was collected on dry ice from rats before and after intraperitoneal injection of rhIL-1. The urinary metabolite of $PGF_2\alpha$ [13,14-dihydro-15ketoPGF$_2\alpha$ (Met$F_2\alpha$)], an indicator of total body PG production, was extracted using a Sep-pak C18 cartridge (Waters Associates, Milford, MA) as described by Powell (26). Met$F_2\alpha$ in the extracts was measured by RIA as described previously (27), and urinary creatinine was measured by Roche Cobas Fara Centrifugal Analyzer (Nutley, NJ) using Roche Diagnostic Systems Reagent and procedure number 44905, a modification of the method of Larson (28).

Rats were anesthetized with metaphane and peritoneal leukocytes were obtained by lavaging the peritoneal cavity with ~ 50 ml of Ca^{2+} and Mg^{2+} free PBS solution. The cell pellet was obtained by centrifugation at 1,200 rpm for 10 min. Cells were washed two times in RPMI 1640 (Gibco Laboratories, Grand Island, NY) and resuspended in RPMI 1640 at a concentration of 5×10^6 cells/ml. 100 μl of cell suspension (5×10^5 cells) was cultured in 96-well culture plates (Becton-Dickinson Co., Oxnard, CA) in the presence of 0.5% fetal bovine serum (Gibco Laboratories) and 50 μl of 0.1% BSA (Sigma Chemical Co., St. Louis, MO) or 50 μl of 60 ng/ml rhIL-1β solution in 0.1% BSA (final rhIL-1β concentration 15 ng/ml) for 24 h in a 37°C, 5% CO_2 humidified incubator. Cell-free supernatant was saved at −70°C for PGE_2 analysis. PGE_2 was analyzed by RIA (27). PGE_2 antibody had a cross-reactivity of 5.6% with PGE_3 standard (Cayman Chemical Co., Ann Arbor, MI). Blood was collected from the vena cava and brain dissected on an ice-cold platform and immersed with ice-cold buffer. The mid-brain (including hypothalamus, striatum, and hippocampus) and brain stem (including medulla oblongata and pons) were dissected with precooled tools. 10% homogenates from brain regions and liver were incubated for 30 and 10 min, respectively, in a shaking water bath at 37°C. PGE_2 was measured in the supernatant of the incubation media. Student's *t* test or one-way ANOVA (when significant, followed up by Tukey-HSD procedure with a procedure-wise error rate of 0.05) was used to determine significance of differences between the means of the dietary groups. When unequal variances were present, logarithmic transformation of the data was performed. If this resolved the unequal variances, statistics were performed on the transformed data.

Results

First, the anorexigenic dose of intraperitoneal rhIL-1β in meal-fed rats was established. Administration of 0.1% serum, alone or with 80 ng (400 ng/kg) or 240–400 ng (1.2–2.0 μg/kg) of rhIL-1β i.p. 1 h before the daily meal had no effect on food intake in young (200 g) chow fed rats (Fig. 1). Administration of 800 ng (4 μg/kg) rhIL-1β had a marked anorexic effect (Fig. 1), decreasing food intake by 37.5±6.6% ($P < 0.005$) in this set of rats. Food intake increased to 97.0±3.3% of baseline values by the next day (NS vs. baseline). Boiled rhIL-1β given at 4–6 μg/kg i.p. had no anorexic effect. Because many, but not all of the diverse effects of IL-1β are mediated by PGE_2 (29), we administered the cyclooxygenase inhibitor ibuprofen 10 mg/kg i.v. 10 min before injection of 800 ng rhIL-1β i.p. This completely blocked the acute anorexic effect of rhIL-1β (Fig. 1). Acetaminophen, which preferentially inhibits brain cyclooxygenase relative to that in peripheral tissues (30, 31), did not block rhIL-1β anorexia (not shown).

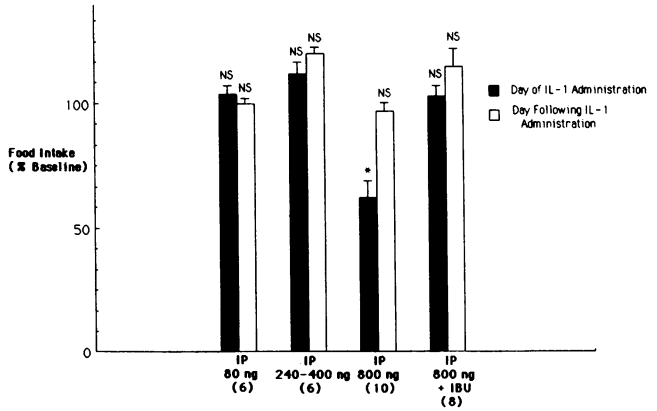

Figure 1. Effect of intraperitoneal rhIL-1β alone and with intravenous ibuprofen on food intake in meal-fed rats. Rats weighing 175–225 g were studied. Housing was in individual cages in a temperature controlled room (22°C) with a 12-h dark/light cycle daily. Catheterization may depress food intake for 24–48 h, therefore no studies were performed for several days after catheter placement, until food intake had stabilized at precatheterization values. RhIL-1β was administered intraperitoneally 1 h before the daily meal. Baseline food intake represents the mean±SEM daily intake of the previous 7–10 d. *P* values are in comparison to baseline food intake, using a two-tailed *t* test. Ibuprofen (IBU) was administered at a dose of 10 mg/kg i.v., 10 min before administration of rhIL-1β. *$P < 0.005$.

Different structural forms and species sources of recombinant IL-1 were also tested. RhIL-1α was less anorexigenic than rhIL-1β in chow-fed rats (although this did not reach statistical significance), while the effect of rmIL-1α was similar to that of rhIL-1β (Table I). Anorexia from intraperitoneal rmIL-1α, as from intraperitoneal rhIL-1β, was completely blocked by ibuprofen 10 mg/kg i.v. (not shown). To exclude any possible effects due to the intravenous route of ibuprofen administration or due to catheterization itself, ibuprofen 10 mg/kg or 0.9% saline were administered to noncatheterized rats by the intraperitoneal route 1 h before rmIL-1α 6 μm/kg i.p., (Fig. 2). IP ibuprofen completely abolished rmIL-1α anorexia (food intake 99.4% with ibuprofen/IL-1, 56.3% with saline/IL-1).

Next, the effect of dietary fat source on IL-1 anorexia was examined (Table I). Exogenous N-3 fatty acids, which are present at high levels in cold water F.O. (24) are known to reduce PGE_2 synthesis in rats (32) as well humans (33). Groups of rats were chronically fed chow or purified diets based on AIN diet recommendations for rats (34) containing 10% by weight of either F.O. or C.O. Animals were begun on purified diets at 75–100 g body wt and continued for up to 6–10 wk. Composition of tissue fat in growing rats has been shown to be altered by dietary fat source (35). Serum tocopherol levels in these F.O., C.O. and chow fed rats were comparable (not shown). The effect of rhIL-1β 4 μg/kg or rmIL-1α 6 μg/kg injections on food intake was significantly different between the F.O. and C.O. groups with chow-fed rats in between (Table I). Administration of 0.9% saline i.p. had no effect on food intake in any group. The effect of dietary fat source was similar for rmIL-1α as for rhIL-1β (Table I). To test the hypothesis that this nutrient-nutrient interaction (prior dietary fat source influencing food intake response to IL-1 administration) is mediated by eicosanoid pathways, PGE_2 synthesis was determined in vitro and in vivo in basal and IL-1β stimulated states. Consistent with the model, in vivo production of PGs of the 2 series, represented by the urinary excretion of the $PGE_2\alpha$ metabolite 13,14-dihydro-15keto-$PGF_2\alpha$ ($MetF_2\alpha$) was higher in C.O. fed rats after rhIL-1β injection than in F.O. fed rats (Table II). Moreover, peritoneal leukocytes from C.O. fed rats synthesized more PGE_2 than F.O. fed rats in vitro, both in the presence and absence of rhIL-1β (Table III). Brain stem and liver homogenates from F.O. fed rats also produced significantly less PGE_2 than C.O. and chow fed rats (Table III), although no such difference was observed in mid-brain.

When rhIL-1β was given intravenously as a 800-ng (4 μg/kg) bolus 1 h before the daily meal, there was no effect on food intake (Table IV). The same dose administered by constant intravenous infusion at 200 ng/h over 4 h beginning 1 h before the meal reduced food intake by 34.8±15.9% ($P < 0.005$), which was not significantly different from equal doses given intraperitoneally. When rhIL-1β was infused overnight at 80 ng/h for the 20 h before and during the daily

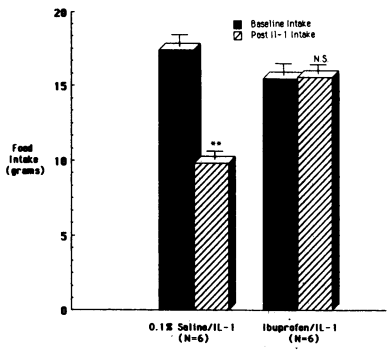

Figure 2. Effect of pretreatment with intraperitoneal ibuprofen (10 mg/kg) or 0.9% saline 1 h before administration of rmIL-1α 6 μg/kg on food intake in chow fed rats. Ibuprofen or 0.9% saline were given in 1 ml volume. Baseline values were calculated as the mean intake for the previous 9 d for each animal. Values shown as mean±SE for each group. *P* values are calculated by two-tailed *t* test. $^{**}P < 0.01$; NS, not significant.

Table I. Effect of Dietary Fat Source and Recombinant IL-1 Structural Form and Species Source on rIL-1 Induced Anorexia

Dietary group	IL-1 form	Effect on food intake
	n	*% Decrease Mean±SE*
Chow	rhIL-1β (12)	18.9±3.2%‡
	rmIL-1α (41)	23.6±3.2%‡
	rhIL-1α (16)	12.7±3.6%
Corn oil	rhIL-1β (12)	32.1±5.3%‡
	rmIL-1α (12)	44.8±5.5%*
	rhIL-1α (5)	8.5±4.6%
Fish oil	rhIL-1β (11)	6.4±6.5%
	rmIL-1α (12)	4.9±4.6%
	rhIL-1α (6)	−1.1±5.3%

Rats were fed fish oil, corn oil, or chow diets as described in the text. RhIL-1β 4 μg/kg, rmIL-1α 6 μg/kg, or rhIL-1α 6 μg/kg i.p. were administered 1 h before the daily meal. Changes in food intake were calculated by comparison to the animal's mean daily intake over the preceding 7 d. *P* values are calculated using one-way ANOVA followed up by Tukey-HSD procedure when significant. *P* value for overall effect of dietary fat or IL-1 source on food intake was 0.0001 by one-way ANOVA.

* $P < 0.05$ vs. all other groups.

‡ $P < 0.05$ vs. fish oil fed (all IL-1 sources).

Table II. Urinary $MetF_{2\alpha}$ Concentration in Rats Fed Different Diets before and after Intraperitoneal rhIL-1β Injection (Mean±SEM, n = 6)

	$MetF_{2\alpha}$ (ng/ng creatinine)	
Diet	Before injection	After injection
Fish oil	0.33±0.02	0.22±0.04*
Corn oil	0.35±0.05	0.52±0.16
Chow	0.35±0.06	0.40±0.15

* $P < 0.09$ vs. corn oil fed rats.

48 h urine was collected on dry ice from rats before and after intraperitoneal injection of rhIL-1β. Logarithmic transformation of data was used when there were unequal variances, and one-way ANOVA was then applied with Tukey's followup.

Table III. PGE_2 Synthesis by Peritoneal Leukocytes and Liver and Brain Stem Homogenates of Rats Fed Different Diets in the Presence or Absence of rhIL-1β (Mean±SEM)

Diet	Peritoneal macrophages		Liver	Brainstem
	Control (*n*)	+rhIL1β (*n*)	(*n*)	(*n*)
	pg/5 × 10⁶ cells		*ng/g*	
Fish oil	220±52 (4)	328±73* (5)	42±7‡ (6)	9±1§ (5)
Corn oil	941±440 (5)	1327±493 (5)	329±72 (6)	18±2 (5)
Chow	—	—	304±63 (5)	19±3 (5)

Tissues were isolated as described in the text. PGE_2 was measured by RIA (26) in the supernatant of the incubation media. *P* values are calculated using two-sample Student's *t* test for peritoneal macrophage data (two groups) and one-way ANOVA for liver and brainstem data (three groups). Statistics were performed on logarithmically transformed data to resolve unequal variances.
* $P < 0.09$ vs. corn oil fed.
‡ $P < 0.0001$ for overall diet effect by ANOVA. Fish oil significantly different from corn oil and chow ($P < 0.05$) by Tukey's followup.
§ $P < 0.0012$ for overall diet effect by ANOVA. Fish oil significantly different from corn oil and chow ($P < 0.05$) by Tukey's followup.

meal (1,600 ng total, Table IV), food intake was markedly decreased, and remained decreased the next day in comparison to preceding and succeeding intakes while catheterized. Inclusion of ibuprofen at 2.5 mg/kg per h to the overnight intravenous rhIL-1β infusion partially restored food intake toward baseline values (Table IV), but not completely. RmIL-1α administered at 80 ng/h (400 ng/kg per h) for 20 h also decreased food intake (to 66% baseline) and this was blocked by co-administration of ibuprofen 2.5 mg/kg per h (intake 108% baseline).

Table IV. Effect of Intravenous rhIL-1β Alone and with Intravenous Ibuprofen on Food Intake (g/d) in Meal-fed Rats

Group (*n*)	Baseline food intake	Day 1 intake (% Baseline)	Day 2 intake (% Baseline)
I Bolus 800 ng i.v. (4)	11.0±1.0	12.4±1.8 (113.0±16%)	— —
II Constant infusion at 200 ng/h × 4 h (7)	13.2±1.8	8.6±2.1‡ (65.2±15.9%)	13.6±0.7 (103.0±5.0%)
III Constant infusion at 80 ng/h × 20 h (6)	11.1±0.6	1.3±1.2‡ (11.7±10.8%)	6.1±1.9‡ (55.0±17.1%)
IV Constant infusion at 80 ng/h × 20 h + i.v. ibuprofen 2.5 ng/kg per h × 20 h (5)	12.7±1.0	6.2±1.6‡§ (48.8±12.6%)	8.6±1.9* (67.7±14.9%)

rhIL-1β was administered intravenously to chronically catheterized rats. Bolus refers to a single injection 1 h before the daily meal. Constant infusions were performed as detailed in the text, using a Harvard infusion pump. Baseline, day one and day two food intakes and *P*-values for comparison were calculated as described in Fig. 1.
* $P < 0.025$ vs. baseline.
‡ $P < 0.005$ vs. baseline.
§ $P < 0.025$ vs. group III, day 1 intake.

The question whether normal aging alters the anorexic effect of rhIL-1β was addressed, because many normal homeostatic responses are diminished with aging (36) and because there exists some evidence (Hellerstein, M. K., unpublished observations) that elderly humans are less able to mount other features of the acute-phase response to inflammation (e.g., fever, leukocytosis, etc.). NIA aged, 18–20-mo old chow-fed Fisher 344 rats had diminished sensitivity to IL-1β anorexia (8.3±3.9% inhibition of food intake, $P < 0.05$ vs. young chow-fed rats).

Finally, we examined the effect of chronic IL-1 administration on food intake and body weight in rats fed different fat sources. These experiments consume large amounts of recombinant protein. Accordingly, only a relatively small number of animals could be studied (5 chow rats, 3 F.O., 3 C.O.). To minimize the possible confounding effects of antibodies to a foreign protein developing with chronic administration in this rodent model, rmIL-1α was given. Rats were given daily rmIL-1α 6 μg/kg or 0.1% serum i.p. Average food intake over the first 7 d of IL-1 administration was significantly diminished in chow-fed rats (16.6±1.0 to 13.5±0.9 g/d, a decrease of 18.7%) and C.O.-fed rats (19.3±2.6 to 13.4±2.3 g/d, a decrease of 30.6%) but not in F.O.-fed rats (14.2±1.9 to 13.7±2.6 g/d, a decrease of 3.5%) (Fig. 3). IL-1α administration was continued in the C.O. and chow-fed groups beyond the initial 7 d. The anorexic effect in both groups was attenuated during days 8–17 of IL-1 administration (Fig. 3), and there was no apparent anorexic effect days 18–25 of IL-1 administration (done in chow group only). After discontinuation of IL-1 treatment (days 18–25 for C.O., 26–32 for chow), food intake returned to baseline levels in both groups (chow 99.4%, C.O. 97.9%). The chow group was rechallenged with IL-1 on days 33–40 and

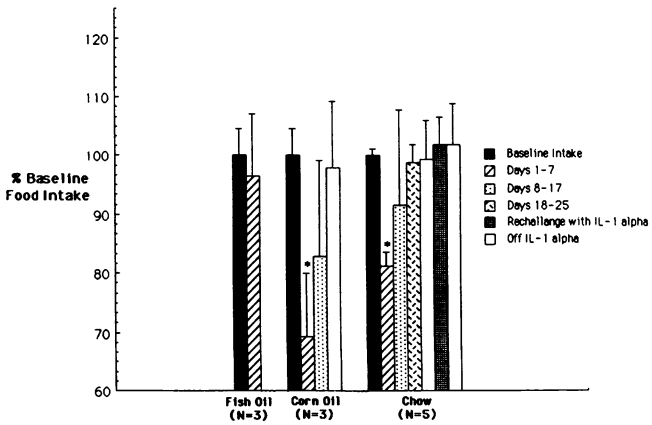

Figure 3. Effect of chronic rmIL-1α administration on food intake in fish oil, corn oil, and chow-fed rats. A dose of 6 μg/kg i.p. was given daily. *P* values calculated by one-way ANOVA, which revealed an interaction between diet and time on effect of IL-1 (e.g., diet alters pattern over time, $P < 0.03$). *$P < 0.05$ vs. baseline intake. Each bar represents the mean±SE from 21–50 data points (three to five animals × 7–10 d of rmIL-1α).

there was no anorexic effect observed (intake 101.8% with or without IL-1). Body weights for chow fed rats given IL-1α are shown in Fig. 4. Initially, weight gain is entirely prevented but after 7–10 d weight gain is again observed, though the slope of gain is less than it was before rmIL-1α administration and is less than in paired rats (average weight gain during IL-1 administration was 1.5±0.2 g/d, $P < 0.05$ vs. pre-IL-1 weight gain [3.6±0.2 g/d] and $P < 0.05$ vs. control group weight gain [2.7±0.2 g/d]). Similar results were observed in the C.O.-fed group (not shown).

Discussion

These results demonstrate that the meal-fed rat is a useful animal model for quantifying the effects of purified mediators on food intake and that rhIL-1β and rmIL-1α reduce food intake in the rat at doses ≥ 4 μg/kg i.p. PGE_2 production appears to be required for the IL-1 effect, based on the ability of intravenous or intraperitoneal ibuprofen to block the effect completely, the ability of chronic F.O. feeding to markedly decrease it, and the observation that F.O. fed rats have significantly lower in vivo and in vitro PGE_2 production. The anorexic effect of rhIL-1β and rmIL-1α were similarly modulated by F.O. feeding and ibuprofen treatment.

The interaction between IL-1 secretion and PGE_2 production is complex. PGE_2 may inhibit further IL-1 secretion by endothelial cells and macrophages (37), representing a short negative feedback loop. Since not all the actions of IL-1 are mediated by PGE_2 (e.g., certain immunostimulatory effects, reference 29), inhibition of macrophage and other tissue PGE_2 production (Table III) may increase IL-1 secretion and non-PGE_2 mediated IL-1 effects, even as it decreases those mediated by PGE_2. Proper classification of IL-1 anorexia, a presumably undesirable effect in the clinical setting, as PGE_2 mediated is therefore important. It is worth noting that cyclooxygenase inhibition also prevents a number of effects of TNF (38, 39) and endotoxin (39) without preventing the endotoxin-induced rise in circulating TNF (39).

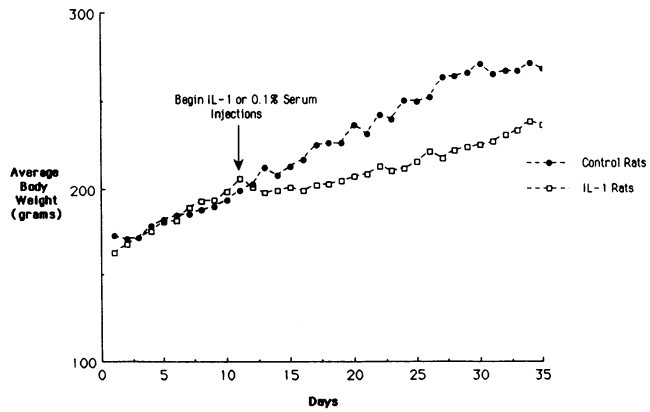

Figure 4. Effect of chronic rmIL-1α administration on body weight in chow-fed rats. A dose of 6 μg/kg i.p. was given daily. Average weight gain of IL-1 group was 1.5±0.2 g/d during IL-1 administration, $P < 0.05$ vs. weight gain pre-IL-1 (3.6±0.2 g/d) and $P < 0.05$ vs. control group (2.7±0.2 g/d), by two-tailed t test.

Comparisons with previous studies of the effect of IL-1 on food intake in the rat are complicated by methodologic considerations. The report of McCarthy et al. (40) that intracerebroventricularly administered endotoxin and "IL-1" induced fever but did not suppress intake of a liquid diet in previously fasted rats must be interpreted with caution, since human monocyte supernatant containing IL-1 was administered, not recombinant IL-1. The brief and relatively minor effect of rmIL-1α on intake of a liquid diet reported in the rat (14) in comparison to the more robust effects on total daily intake shown here with rhIL-1β and rmIL-1α has several possible explanations. The liquid diet is not a physiologic form of food and may modify any effects of IL-1 on appetite that are mediated by suppression of gastric motility (41). Due to rapid clearance of administered IL-1β (37), the meal-feeding model may be superior for revealing IL-1β anorexia. The increased hepatic lipogenesis of meal-fed rats (19–21) may also increase sensitivity to IL-1 anorexia, if the mechanism is a peripheral one and involves alterations in hepatic carbohydrate utilization or triose metabolism, as appears to underlie several other metabolic models of anorexia (17, 18, 42–46). The ability of cytokines to stimulate hepatic lipogenesis (47) in the rat is suggestive in this regard. The difference is unlikely to be due to the form of IL-1 used, since we (Table I) and Moldawer et al. (16) have found rmIL-1α to be an effective anorexigen in rodents.

Our observation of differences between rhIL-1β and rhIL-1α (Table I) is consistent with other reports of different activities of IL-1α and β in some systems (e.g., 48) and may reflect different affinities of IL-1α and IL-1β for the IL-1 receptor, and different abilities to generate PGE_2. The difference between rhIL-1α and rmIL-1α emphasizes the importance of species source. The molecular basis of these variations in actions between IL-1 from different species is unknown, although the profound effect of dietary fat source on both rhIL-1β and rmIL-1α anorexia suggests a shared mechanism for their anorexigenic actions.

The question whether IL-1 anorexia operates by a central or peripheral mechanism can not be definitively answered yet. The failure of intravenous boluses of rhIL-1β (800 ng) to cause any decrease in food intake (Table IV) is probably due to rapid renal clearance of IL-1β (37), in view of the ability of intravenous rhIL-1β or rmIL-1α continuously infused at lower doses to suppress food intake. The inability of acetaminophen, which preferentially inhibits brain cyclooxygenase relative to that in peripheral tissues (30, 31), to block rhIL-1β anorexia supports a peripheral mechanism. The inability of intravenous ibuprofen to completely block the anorexic effect of prolonged, low dose rhIL-1β infusion also suggests that other mechanisms besides PGE_2 production may be involved in this setting, although other explanations are possible since PG production was not measured. These questions require further study.

The finding that normal aging decreases sensitivity to the anorexic effect of rhIL-1β is of interest. One might have expected a greater effect on food intake in these old, presumably less resilient animals. The fact that the opposite was observed indirectly supports the IL-1β effect as being part of a "designed" physiologic response, which is attenuated with aging, in contrast to a model wherein anorexia is a "nonspecific" effect of illness.

By analogy to TNF, the possibility needs to be considered that IL-1 induced suppression of food intake is a toxic effect rather than a primary anorexigenic effect. Daily administration of recombinant human TNF-α to rodents (12) initially caused acute gastrointestinal inflammation, with edema and hemorrhage, followed by rapid recovery within 24–48 h (tolerance). Food intake and weight loss followed a similar time course, with an acute effect followed by rapid tolerance (12). However, IL-1 anorexia is unlikely to be due to acute gastrointestinal toxicity, for several reasons. First, extensive toxicity studies in mice and rats using 100-fold higher IL-1 doses than were used here have failed to reveal any organ toxicity (Dinarello, C. A., unpublished observations). In fact it is quite difficult to kill a nonadrenalectomized rodent with massive doses of IL-1, in contrast to TNF. Second, one would expect gastrointestinal toxicity to be worsened by prostaglandin inhibition, rather than ameliorated, since PGE_2 is cytoprotective in the gut (49, 50). Finally, the anorexia and slowing of body weight gain that we observed were not restricted to the first 24–48 h of IL-1 administration, but persisted for at least 7 d (Figs. 3 and 4), and withholding of IL-1 for a day during chronic administration immediately restored food intake to normal (not shown).

The effects of chronic rmIL-1α administration are notable for several reasons. The issue of tachyphylaxis to cytokine anorexia is central if a physiologic role for cytokines in the anorexia of chronic disease is to be considered tenable. Our observations indicate that tachyphylaxis to IL-1 anorexia (attenuation of anorexigenic effects and return of some weight gain) does occur, but equally importantly show at least a 7-d effect for the anorexia at a constant rmIL-1α dose (Fig. 3) and a net lower weight in the IL-1 treated rats compared to controls after several weeks (Fig. 4). Thus, IL-1 by itself has long-term nutritional consequences when administered chronically, in this model. Also, the dietary fat effects observed in acute food intake experiments were reproduced for at least the first 7 d of treatment, supporting the physiologic relevance of the acute data. The mechanism for tachyphylaxis was not established by these studies (e.g., development of antibodies to the recombinant IL-1, a well-documented event even in the same species, receptor down-regulation, metabolic adaptations, etc.). The question whether synergy with other cytokines (51, 52) would overcome this attenuation is currently under investigation.

Placed in the larger picture, these findings add support to the concept that nutrients and inflammation are intimately intertwined, in a complex bidirectional fashion mediated by cytokines (Fig. 5). Thus, dietary protein and source of fatty acids may alter IL-1 release (53, 54), dietary fats may alter cytokine actions (see above), carbohydrates may in theory be involved as signals in their anorexic effects (18, 42–46), and inflammatory mediators not only alter metabolism of all classes of substrates (47, 55–57) but reduce their overall intake (cause anorexia). Inclusion of IL-1 anorexia in this scheme is now necessary. From a clinical perspective, perhaps most interesting is the ability of a dietary supplement to diminish IL-1 anorexia. This would be a simple and safe intervention in patients experiencing (or expected to experience) anorexia and weight loss in association with an inflammatory illness or injury. If IL-1 is involved in the human syndrome and acts through PGE_2 generation, as is the case in the rat, therapeutic trials of F.O. supplementation may be rewarding. By analogy with F.O. supplementation used for other clinical purposes (58) it is unlikely that humans would need to ingest a diet with 80% of the fat as F.O. to test the hypothesis. Human volunteers adding 3 g/d of EPA in F.O. capsules to their otherwise normal western diets demonstrated a 50% reduction in IL-1 production when their blood leukocytes were stimulated ex vivo (59). From an experimental point of view, the importance of species source and structural form of IL-1 used, dose, chronicity, and route of administration, feeding regimen, dietary fat source and age of animals in determining the effect of IL-1 on food intake needs to be taken into account and specified in future studies.

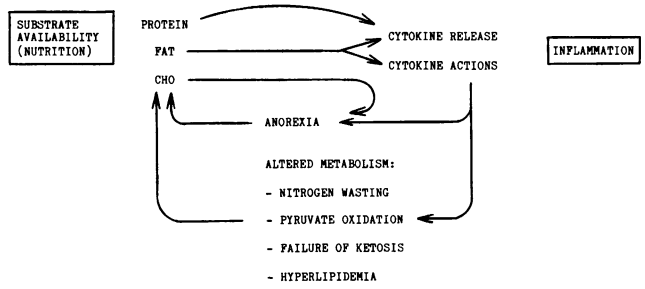

Figure 5. Cytokine-mediated interactions between nutrients and inflammation. See text for details.

Acknowledgments

The authors are grateful to Dr. J. Dupont of Iowa State University and M. M. Mathias of Colorado State University for providing PGE_2 antibody, to Dr. J. D. Davies of R. P. Scherer Co., Troy, MI for supplying MaxEPA, to Denise Cesar and Paul Bizinkauskas for technical assistance, and to Mark Hudes for statistical consultation.

Supported by grant R87SF091, provided by the State of California and allocated on the recommendation of the University of California Universitywide Task Force on AIDS (M. Hellerstein), USDA Contract 53-3K06-5-10 (S. Meydani and M. Meydani), and National Institutes of Health grant AI15614 (C. Dinarello).

References

1. Beisel, W. R. 1975. Metabolic response to infection. *Annu. Rev. Med.* 26:9.

2. Hornstein, I. 1977. Impact of infection on nutritional status (Symposium). *Am. J. Clin. Nutr.* 30:1206–1210.

3. Theologides, A. 1979. Cancer cachexia. *Cancer* 43:2004–2012.

4. Theologides, A. 1986. Anorexins, asthenins and cachectins in cancer. *Am. J. Med.* 81:696–698.

5. Kotler, D. P., J. Wang, and R. N. Pierson. 1985. Body composition studies in patients with acquired immunodeficiency syndrome. *Am. J. Clin. Nutr.* 42:1255–1264.

6. Chlebowski, R. T. 1985. Significance of altered nutritional status in AIDS. *Nutr. Cancer.* 7:85–91.

7. Serwadda, D., N. K. Sewankambo, J. W. Carswell, A. C. Bayley, R. S. Tedder, R. A. Weiss, R. D. Mugerwa, A. L. Wegaba, G. B. Kirya, R. G. Downing, S. A. Clayden, and A. G. Dalgleish. 1985. Slim disease: a new disease in Uganda and its association with HTLV-III infection. *Lancet.* ii:849–852.

8. Dinarello, C. A., J. G. Cannon, J. W. Mier, H. A. Bernheim, G. LoPreste, D. L. Lynn, R. N. Love, A. C. Webb, P. E. Auron, R. C. Reuben, A. Rich, S. M. Wolff, and S. D. Putney. 1986. Multiple biological activities of human recombinant interleukin 1. *J. Clin. Invest.* 77:1734–1739.

9. Beutler, B., and A. Cerami. 1987. Cachectin: more than a tumor necrosis factor. *N. Engl. J. Med.* 316:379–385.

10. Cerami, A., Y. Ikeda, N. LeTrang, P. J. Hotez, and B. Beutler.

1985. Weight loss associated with an endotoxin induced mediator from peritoneal macrophages. The role of cachectin (tumor necrosis factor). *Immunol. Lett.* 11:173–177.

11. Oliff, A., D. Defeo-Jones, M. Bover, D. Martinez, D. Kiefer, G. Vuocolo, A. Wolfe, and S. H. Socher. 1987. Tumors secreting human TNF/cachetin induce cachexia in mice. *Cell.* 50:555–563.

12. Patton, J. S., P. M. Peters, J. McCabe, D. Crase, S. Hansen, A. B. Chen, and D. Liggit. 1987. Development of partial tolerance to the gastrointestinal effects of high doses of recombinant tumor necrosis factor α in rodents. *J. Clin. Invest.* 80:1587–1596.

13. Tracey, K., H. Wei, K. Manogue, G. Kuo, S. F. Lowry, and A. Cerami. 1988. Weight loss, cachexia and inflammation in rats treated with recombinant cachectin. *J. Exp. Med.* 167:1211–1227.

14. Dinarello, C. A. 1989. Interleukin-1 and its biologically related cytokines. *Adv. Immunol.* 44:153–205.

15. McCarthy, D. O., M. J. Kluger, and A. J. Vander. 1985. Suppression of food intake during infections: is interleukin-1 involved? *Am. J. Clin. Nutr.* 42:1179–1182.

16. Moldawer, L. L., C. Anderson, J. Gelin, and K. G. Lundhold. 1988. Regulation of food intake and hepatic protein synthesis by recombinant-derived cytokines. *Am. J. Physiol.* 254:G450–G456.

17. Dinarello, C. A., J. G. Cannon, S. M. Wolff, H. A. Bernheim, B. Beutler, A. Cerami, I. S. Figari, M. A. Palladino, Jr., and J. V. O'Connor. 1986. Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. *J. Exp. Med.* 163:1433–1450.

18. Sullivan, A. C., J. Triscari, J. G. Hamilton, and O. N. Miller. 1973. Effect of (−)-hydroxycitrate upon the accumulation of lipid in the rat: II. Appetite. *Lipids.* 9:129–134.

19. Hellerstein, M. K., and Y. Xie. 1987. Effect of 3-mercaptopicolinic acid on food intake in normal, hydroxycitrate treated, vagotomized and hypoglycemic rats. *Clin. Res.* 35:366a. (Abstr.)

20. Leveille, G. A. 1972. The long-term effects of meal-eating on lipogenesis, enzyme activity and longevity in the rat. *J. Nutr.* 102:549–556.

21. Fabry, P. 1967. Metabolic consequences of the pattern of food intake. *In* Handbook of Physiology, Section 6, Alimentary Canal. Vol. 1. C. F. Code, editor. Am. Physiol. Soc., Washington, DC. 31.

22. Tepperman, J., and H. Tepperman. 1958. Effects of antecedent food intake pattern on hepatic lipogenesis. *Am. J. Physiol.* 193:55–64.

23. Meydani, S. N., A. C. Shapiro, M. Meydani, J. B. MaCauley, and J. B. Blumberg. 1987. Effect of age and dietary fat (fish, corn, and coconut oils) on tocopherol status of C57BL6NIA mice. *Lipids.* 22:345–350.

24. Cathcart, E. S., C. A. Leslie, S. N. Meydani, and K. C. Hayes. 1987. A fish oil diet retards experimental amyloidosis, modulates lymphocyte function and decreases macrophage arachidonate metabolism in mice. *J. Immunol.* 139:1850–1855.

25. Hellerstein, M. K., D. J. Greenblatt, and H. N. Munro. 1986. Glycoconjugates as non-invasive probes of intrahepatic metabolism. The pathways of glucose entry into compartmentalized hepatic UDP-glucose pools during feeding induced glycogen accumulation. *Proc. Natl. Acad. Sci. USA.* 83:7044–7048.

26. Powell, S. S. 1980. Rapid extraction of oxygenated metabolites of arachidonic acid from biological samples using octadecylsilyl silica. *Prostaglandins.* 20:947–957.

27. Meydani, S. N., and J. Dupont. 1982. Effect of zinc deficiency on prostaglandin levels in different organs of the rat. *J. Nutr.* 112:1098–1104.

28. Larson, K. 1972. Creatinine assay by a reaction-kinetic principle. *Clin. Chim. Acta.* 41:209–217.

29. Dinarello, C. A., S. O. Marnoy, and L. J. Rosenwasser. 1983. Role of arachidonate metabolism in the immunoregulatory function of human leukocytic pyrogen/lymphocyte-activating factor/interleukin 1. *J. Immunol.* 130:890–895.

30. Flower, R. J., and J. R. Vane. 1972. Inhibition of prostaglandin synthetase in brain explains the antipyretic activity of paracetamol (4-acetaminophenol). *Nature (Lond.).* 240:410–411.

31. Ziel, R., and P. Krupp. 1976. Mechanism of action of antipyretic drugs. *In* Brain Dysfunction in Infantile Febrile Convulsions. M. A. B. Brazier and F. Coceani, editors. Raven Press, New York. 141–153.

32. Prickett, J. D., D. E. Trantum, and D. V. Robinson. 1984. Dietary fish oil augments the induction of arthritis in rats. *J. Immunol.* 132:725–730.

33. Lee, T. H., R. L. Hoover, J. D. Williams, R. I. Sperling, J. Ravalese, B. W. Spur, D. R. Robinson, E. J. Corey, R. A. Lewis, and K. F. Austen. 1985. Effect of dietary enrichment with eicosapentaenoic acide and docosahexanoic acid on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. *N. Engl. J. Med.* 312:1217–1224.

34. Report of The American Institute of Nutrition. Ad Hoc Committee on Standards for Nutritional Studies. 1977. *J. Nutr.* 107:1340–1349.

35. Sprecher, H., A. C. Voss, M. Careaga, and C. Hadjiagapiou. 1987. Interrelationship between polyunsaturated fatty acid and membrane lipid synthesis. *In* Proceedings of the American Oil Chemists' Society (AOCS) Short Course on Polyunsaturated Fatty Acids and Eicosanoids. W. E. M. Lands, editor. AOCS, Champaign, IL. 154–168.

36. Shock, N. W. 1972. *In* Nutrition in Old Age. L. A. Carlson, editor. Almquist and Wiksell, Uppsala. 12–23.

37. Dinarello, C. A., T. Ikejima, S. J. C. Warner, S. F. Orencole, G. Lonneman, J. G. Cannon, and P. Libby. 1987. Interleukin 1 induces interleukin 1. 1. Induction of circulating interleukin 1 in rabbits in vivo and in human mononuclear cells in vitro. *Am. Assoc. Immunol.* 139:1902–1910.

38. Okusawa, S., J. A. Gelfand, T. Ikejima, R. J. Connolly, C. A. Dinarello. 1988. Interleukin 1 induces a shock-like state in rabbits. Synergism with tumor necrosis factor and the effect of cyclooxygenase inhibition. *J. Clin. Invest.* 81:1162–1172.

39. Michie, H. R., K. R. Manogue, D. R. Spriggs, A. Revhaug, S. O'Dwyer, C. A. Dinarello, A. Cerami, S. Wolff, and D. Wilmore. 1988. Detection of circulating tumor necrosis factor after endotoxin administration. *N. Engl. J. Med.* 318:1481–1486.

40. McCarthy, D. O., M. J. Kluger, and A. J. Vander. 1985. Effect of centrally administered interleukin-1 and endotoxin on food intake of fasted rats. *Physiol. Behav.* 36:745–749.

41. VanMiert, A. 1980. Fever and gastric function. *In* Fever. J. M. Lipton, editor. Raven Press, New York. 57–70.

42. Van Itallie, T. B., N. S. Smith, and D. Quartermain. 1977. Short-term and long-term components in the regulation of food intake: evidence for a modulatory role of carbohydrate status. *Am. J. Clin. Nutr.* 30:742–757.

43. Sullivan, A. C., and R. K. Gruen. 1985. Mechanisms of appetite modulation by drugs. *Fed. Proc.* 44:139–144.

44. Langhans, W., U. Damaske, and E. Scharrer. 1985. Different metabolites might reduce food intake by the mitochondrial generation of reducing equivalents. *Appetite.* 6:143–152.

45. Russek, M. 1981. Current status of the hepatostatic theory of food intake. *Appetite.* 2:137–143, 157–162.

46. Baile, C., W. M. Zinn, and J. Mayer. 1970. Effects of lactate and other metabolites on food intake of monkeys. *Am. J. Physiol.* 219:1606–1613.

47. Feingold, K. R., and C. Grunfeld. 1987. Tumor necrosis factor —alpha stimulates hepatic lipogenesis in the rat in vivo. *J. Clin. Invest.* 80:184–190.

48. Lumpkin, M. D. 1987. The regulation of ACTH secretion by IL-1. *Science (Wash. DC).* 238:452–454.

49. Robert, A. 1975. An intestinal disease produced experimentally by a prostaglandin deficiency. *Gastroenterology.* 69:1045–1047.

50. Robert, A. 1979. Cytoprotection by prostaglandins. *Gastroenterology.* 77:761–767.

51. Movat, H. Z., C. E. Burrowes, M. I. Cybulsky, and C. A. Dinarello. 1987. Acute inflammation and a Schwartzman-like reaction induced by interleukin-1 and tumor necrosis factor. Synergistic

action of cytokines in the induction of inflammation and microvascular injury. *Am. J. Pathol.* 129:463–476.

52. Elias, J. A., K. Gustillo, W. Baeder, and B. Freundlich. 1987. Synergistic stimulation of fibroblast prostaglandin production by recominant interleukin 1 and tumor necrosis factor. *J. Immunol.* 138:3812–3816.

53. Hoffman-Goetz, L., and M. J. Kluger. 1979. Protein deficiency: its effects on body temperature in health and disease states. *Am. J. Clin. Nutr.* 32:1423–1427.

54. Editorial. 1983. Protein malnutrition and leukocyte endogenous mediator. *Nutr. Rev.* 41:242–244.

55. Kampschmidt, R. F. 1981. Leukocyte endogenous mediator/endogenous pyrogen. *In* The physiologic and metabolic responses of the host to infection and inflammation. M. C. Powanda and P. G. Canonico, editor. Elsevier/North Holland, Amsterdam. 55–74.

56. Neufeld, H. A., J. A. Pace, and F. E. White. 1976. The effect of bacterial infections on ketone concentrations in rat liver and blood and on free fatty acid concentrations in rat blood. *Metab. Clin. Exp.* 25:877–884.

57. Tredget, E. E., Y. M. Yu, S. Zhong, R. Burini, S. Okusawa, J. A. Gelfand, C. A. Dinarello, U. R. Young, and J. F. Burke. 1988. Role of interleukin 1 and tumor necrosis factor on energy metabolism in rabbits. *Am. J. Physiol.* 255:E760–E768.

58. Kremer, J. M., W. Jubiz, A. Michalek, R. Rynes, L. Bartholomew, J. Bigaouette, M. Timchalk, D. Beeler, and L. Lininger. 1987. Fish-oil fatty acid supplementation in active rheumatoid arthritis. A double-blinded, controlled cross-over study. *Ann. Intern. Med.* 106:497–503.

59. Endres, S., R. Ghorbani, V. E. Kelley, K. Georgilis, G. Lonnemann, J. W. M. Van Der Meer, J. G. Cannon, T. S. Rogers, M. S. Klempner, P. C. Weber, E. J. Schaefer, S. M. Wolff, and C. A. Dinarello. 1989. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N. Engl. J. Med.* 320:265–271.