tornadic-intensity vortex formation. On Mars in the late summer, the occurrence of atmospheric instability, a deep convective layer, and onset of baroclinic wave passage represent a similar situation. Could martian conditions lead to formation of similarly intense vortices and therefore the observed lineations? Extended convective uplift of unstable surface air on Mars can occur in the deep convective layer with or without the addition of small amounts of latent heat that would be released by water condensation. As recently reviewed (*4*), high winds associated with baroclinic wave passage result in large vertical shear over low-relief surfaces and the formation of horizontal vortex tubes. Interaction between a sustained convective updraft and the vortex tubes can result in vertical tilting of the vortex tubes. If the relative winds veer with height, vorticity can become parallel to the relative flow and result in the formation of a midlevel mesocyclone, which then can build down to the surface and strengthen. It is believed that, on Earth, this process, driven by latent heat release rather than extended dry convection, leads to tornadogenesis (*4*). On Mars, a paucity of images in desired locations may explain the absence of lineations during similar conditions in the early spring. Because such vortices are controlled by processes well above the surface, they are relatively insensitive to topography and may exhibit large nontopographically initiated gaps in their paths. The rapid disappearance of the lineations by midfall may be the result of burial by dust either from atmospheric fallout or associated with the expanding polar cap.

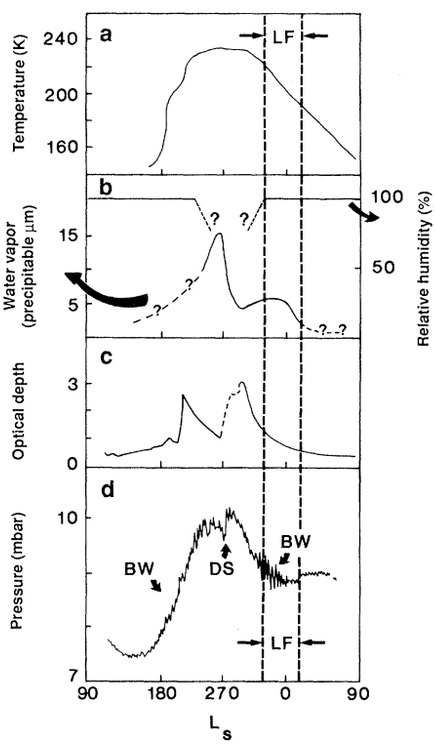


**Fig. 4.** Atmospheric conditions present at 45°S through a martian year. $L_s$ refers to the aerocentric longitude of the sun, with $L_s$ 270 being the summer solstice and $L_s$ 90 being the winter solstice in the southern hemisphere. Lineations form in the period between the dashed lines labeled LF. (**a**) Surface temperatures from (*10*) based on a model developed by H. H. Kieffer of the U.S. Geological Survey. (**b**) Atmospheric water vapor present and corresponding relative humidity, from (*10*) as determined by B. M. Jakosky and from (*14*), respectively. (**c**) Atmospheric optical depth from (*10*) based on data analyzed by J. B. Pollack and other groups. (**d**) Surface record of atmospheric pressure at Viking Lander 2 (226°W, 48°N) from (*17*). Occurrence of baroclinic waves (BW) and the second dust storm of 1977 (DS) are indicated.

Although the seasonal effects of such vortices will be small, they would be significant over time in the absence of other processes and climatic changes. Thus, intense vortices may contribute to the stripping of the northern martian plains and the accumulation of coarse materials inferred to comprise the circumpolar and crater floor dune fields, but the low albedo of much of the northern martian plains may prevent easy detection. On Earth, tornadic-intensity vortices commonly leave distinctive tracks whose appearance is similar to that of the martian lineations (*4*). A high-resolution imaging system as proposed for the Mars Observer mission could resolve these ground tracks, thereby providing indirect evidence of such phenomena and revealing their importance over time.


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# Fish Oil Prevents Insulin Resistance Induced by High-Fat Feeding in Rats


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**Non–insulin-dependent diabetes mellitus is an increasingly prevalent disease in Western and developing societies. A major metabolic abnormality of non–insulin-dependent diabetes is impaired insulin action (insulin resistance). Diets high in fat from vegetable and nonaquatic animal sources (rich in linoleic acid, an ω-6 fatty acid, and saturated fats) lead to insulin resistance. In rats fed high-fat diets, replacement of only 6 percent of the linoleic ω-6 fatty acids from safflower oil with long-chain polyunsaturated ω-3 fatty acids from fish oil prevented the development of insulin resistance. The effect was most pronounced in the liver and skeletal muscle, which have important roles in glucose supply and demand. The results may be important for therapy or prevention of non–insulin-dependent diabetes mellitus.**


REDUCED POTENCY OF INSULIN ACTION, or insulin resistance, is a feature of non–insulin-dependent diabetes mellitus (*1*). The influence of diet on insulin action in target tissues such as muscle, adipose, and liver is poorly understood, but the high fat intake in the Western style diet is considered a major contributor to a number of disease states. These include not only diabetes but also heart disease and obesity (*2*), and direct links between insulin


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**Table 1.** Effects of the three diets on insulin and glucose concentrations and insulin action in epididymal white adipose and interscapular brown adipose tissues. Abbreviations: $R_g'$, glucose metabolic index. Data are presented as mean ± SEM.

| Diet | Insulin concentration (mU/liter) | | Basal glucose concentration (mmol/liter) | $R_g'$ (μmol/100 g · min) | | | |
|---|---|---|---|---|---|---|---|
| | | | | White adipose | | Brown adipose | |
| | Basal | Infusion | | Basal | Infusion | Basal | Infusion |
| Chow | 26 ± 5 | 135 ± 12 | 4.2 ± 0.1 | 0.55 ± 0.11 | 2.30 ± 0.44 | 6.9 ± 2.0 | 58.0 ± 6.5 |
| Fat | 30 ± 3 | 143 ± 10 | 4.2 ± 0.1 | 0.42 ± 0.08 | 2.30 ± 0.21 | 2.1 ± 0.2 | 38.9 ± 4.7 |
| Fish | 35 ± 4 | 116 ± 7 | 4.4 ± 0.2 | 0.65 ± 0.09 | 1.53 ± 0.18 | 7.3 ± 2.6 | 42.7 ± 8.2 |

resistance, diabetes, and the development of obesity have been suggested (*3, 4*). The type of dietary fat is thought to be important in this regard, and much work has focused on saturated compared to unsaturated fats (*5*). Recently, great interest has been generated by studies showing a reduced incidence of heart disease in men eating as little as 30 g of fish per day (*6*). Further, fish oils (which are high in long-chain polyunsaturated ω-3 fatty acids) have a hypolipidemic effect in normal subjects (*7*) and result in a marked lowering of triglycerides in the blood of subjects with type IIb and type V hypertriglyceridemia (*8*). The close relation between hyperlipidemia and insulin resistance and the surprisingly low prevalence of non–insulin-dependent diabetes in Eskimos (*9*), whose diet consists largely of fish, led us to examine whether long-chain polyunsaturated ω-3 fatty acids can protect against the development of impaired insulin action.

Earlier we showed that feeding rats diets high in fat (safflower oil) leads to a major and widespread impairment of insulin action compared to feeding them high-carbohydrate diets (*4, 10*). We now report that replacing as little as 6% of the linoleic ω-6 fatty acids from safflower oil with long-chain polyunsaturated ω-3 fatty acids from fish oil can prevent the development of insulin resistance in rats.

Insulin action was assessed by the euglycemic clamp technique combined with bolus administration of [$^3$H]2-deoxyglucose and [$^{14}$C]glucose to determine insulin-mediated glucose metabolic rate and glycogen synthesis rate in individual tissues (*10–14*). In the present experiments, 36 adult male Wistar rats (60 ± 2 days of age) that were reared on laboratory chow under controlled conditions (21° ± 1°C, 12:12 lighting cycle with lights on at 0600) were randomly chosen and assigned to three groups of 12. (All data are reported as mean ± SEM.) These were fed for 31 ± 1 days (i) on laboratory chow (Allied) containing (in percentage of calories) 65% carbohydrate, 12% fat, and 23% protein (chow group); (ii) on a high-fat diet containing 59% fat (from safflower oil), 10% carbohydate, and 21% protein (fat group); or (iii) on the high-fat diet but with 20% of the safflower oil replaced with extracted tuna oil (fish group). The tuna oil contained 13% eicosapentaenoic acid and 16% docosahexaenoic acid, the two main ω-3 fatty acids by analysis with gas-liquid chromatography. The actual change from the fat to fish diets thus represented the replacement of approximately 6% of the safflower fatty acids with long-chain polyunsaturated ω-3 fatty acids. The intakes of the fat and fish groups were equivalent at 408 and 412 kJ per day, respectively, which was substantially more than that of the chow group (315 kJ per day).

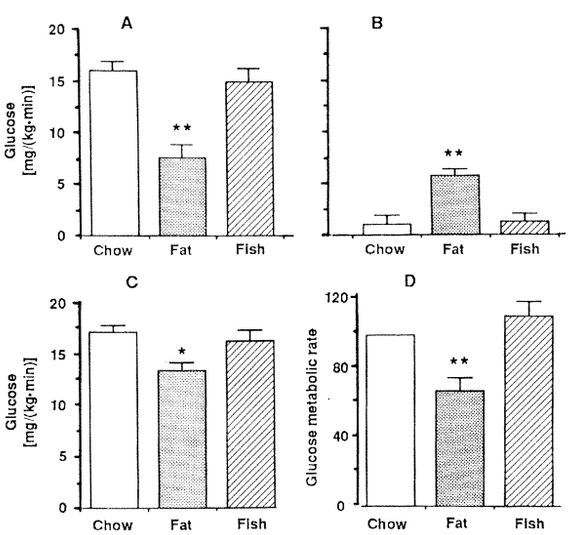


**Fig. 1.** Comparison of the effects of the different diets on measures of insulin-stimulated glucose metabolism. (**A**) The hyperinsulinemic-euglycemic clamp GIR as a measure of overall whole-body insulin action. (**B**) The $R_a$ component of the whole-body GIR. (**C**) The $R_d$ component of the whole-body GIR. (**D**) Overall effects in hind-limb skeletal muscle (average of five individual muscles) expressed as percentage change from the response in the chow group. $*P < 0.05$, $**P < 0.01$, fat compared to both chow and fish groups (data shown as mean ± SEM; $n = 6$). Chow and fish groups did not differ significantly on any of the above measures.

After 29 ± 1 days on their respective diets, rats were anesthetized with intraperitoneal injections of pentobarbitone (30 mg per kilogram of body weight) and intramuscular injections of ketamine hydrochloride (25 mg per kilogram) and then fitted with permanent jugular and carotid cannulas (*10–12*). Studies were conducted in unrestrained, conscious rats 48 hours after surgery, which was sufficient time for the rats to attain near normal food intake and to regain their preoperative weight. At the time of study, individuals in the chow group weighed 365 ± 6 g, those in the fat group weighed 373 ± 5 g, and those in the fish group weighed 385 ± 7 g.

Euglycemic hyperinsulinemic maintenance (clamping) was performed on rats that had been deprived of food for 5 to 6 hours. Briefly, a 2-hour continuous infusion of porcine insulin (Actrapid) was administered through the jugular cannulas at a dose of 4.1 mU/kg · min. Arterial blood glucose was clamped at the basal fasting concentration with a variable glucose infusion rate (GIR). During the second hour of the clamp, the GIR was the steady-state whole-body net glucose disposal rate [peripheral glucose disposal ($R_d$) minus hepatic glucose production ($R_a$)]. Blood was obtained for determination of insulin concentrations in all clamp studies at 0, 60, and 120 minutes. Similar studies at basal insulin concentrations were performed without insulin or glucose infusion.

Insulin action in individual tissues in vivo was studied as described (*10–12*). The nonmetabolizable glucose analog 2,6-[$^3$H]-2-deoxyglucose (2-DG, 50 μCi) was administered together with D-[U-$^{14}$C]-glucose (30 μCi) as an intravenous bolus 75 minutes after the study began. Blood samples for determination of glucose concentrations in blood and radiolabeled glucose and 2-DG in plasma were obtained 2, 5, 10, 15, 20, 30, and 45 minutes after the bolus was administered. At the completion of the clamp, rats were anesthetized with an intravenous injection of pentobarbitone (60 mg per kilogram), and the following tissues were rapidly removed and frozen for subsequent analysis: soleus, red and white gastrocnemius, extensor digitorum longus and plantaris hind limb muscles; diaphragm, lung, heart, epididymal, inguinal, and subcutaneous white adipose tissues; and interscapular brown fat. The muscles were representative of the range of skeletal muscle fiber types (*13*).

An estimate of tissue glucose metabolic rate (the glucose metabolic index, $R_g'$) was calculated from the tissue accumulation of

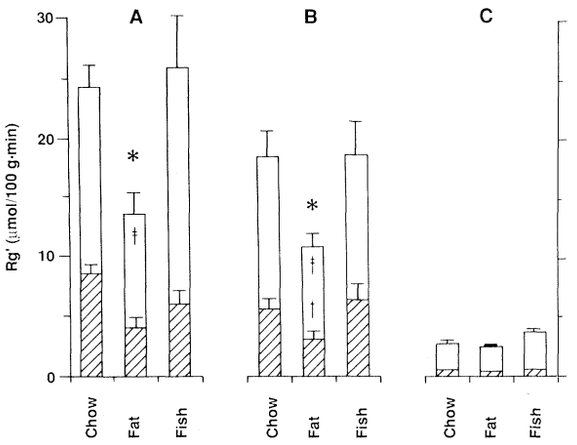


**Fig. 2.** Influence of fish oil on insulin-mediated glucose uptake and disposal in three hind-limb muscles of different fiber types (mean ± SEM; $n = 6$). The entire histogram represents total glucose metabolic rate ($R_g'$), which is divided into the lower hatched portion representing glycogen accumulation and the open upper portion representing net glycolytic flux. Slow-twitch oxidative fiber predominates in soleus (**A**), fast-twitch oxidative-glycolytic fiber predominates in red gastrocnemius (**B**), and fast-twitch glycolytic fiber predominates in white gastrocnemius (**C**). Symbols (fat compared to both chow and fish groups): $*P < 0.01$, $R_g'$; $\dagger P < 0.05$, glycogen accumulation; $\ddagger P < 0.05$, net glycolytic flux.

labeled phosphorylated 2-DG (*11*). In addition, samples of soleus and red and white gastrocnemius were taken for measurement of [$^{14}$C]glucose incorporation into glycogen (*12, 14*). Since [$^{14}$C]glucose incorporation into muscle lipids is negligible (*12*), the simultaneous measurement of $R_g'$ and glycogen accumulation in these muscles enabled us to determine the storage and nonstorage components of glucose utilization (*15*). $R_g'$ minus the storage figure approximates the net sum of glucose oxidation and conversion to three-carbon intermediates and is termed the net glycolytic flux (*14*).

Statistical analysis was by analysis of variance (ANOVA) with Neuman-Keuls post hoc determinations of individual differences or by $t$ test for pair-wise comparisons.

Net whole-body glucose disposal is shown in Fig. 1A. High-fat feeding greatly reduced the GIR by 52%. The fish group showed no diminution in insulin action; this effect was not significantly different from that in the chow group. The improvement in insulin-stimulated glucose disposal in the fish group compared to the fat group could reflect the contribution of improved insulin-mediated suppression of $R_a$ and improved $R_d$. To elucidate the extent of these contributions, we calculated $R_a$ and $R_d$ from the plasma disappearance curves of [$^{14}$C]glucose (*13*) [Fig. 1, B and C (*16*)]. With the usual interpretation given to $R_a$ and $R_d$ (*17*), we conclude that fish oils have approximately equal beneficial effects on insulin action in the liver and peripheral tissues.

Basal insulin and glucose concentrations were essentially unaffected by the different diets (Table 1). Basal glucose turnover was also not significantly affected. Consistent with this, there were no significant differences in $R_g'$ between the three groups in 10 of 12 tissues investigated. The diet for the fat group significantly reduced $R_g'$ only in soleus muscle and interscapular brown adipose tissue; the diet for the fish group reversed the latter but not the former effect. The constant infusion of insulin during the euglycemic clamp raised insulin concentrations marginally less in the fish group. Thus the beneficial effects of the fish oils were achieved even at somewhat lower insulin concentrations.

Analysis of the pattern of $R_d$ in individual peripheral tissues showed that the major effects of the diets were in skeletal muscle (Fig. 1D). The ANOVA results showed a significant ($P < 0.01$) reduction in $R_g'$ in skeletal muscle in the fat group; this effect was completely eliminated in the fish group. Among individual skeletal muscles the pattern was similar. In agreement with earlier findings (*12, 14*), however, those muscles containing predominantly oxidative fiber types (for example, soleus and red gastrocnemius) showed the greatest change in insulin sensitivity. Using the combination of [$^{14}$C]glucose and [$^{3}$H]2-DG, we separated the tissue-specific glucose metabolism into net glycolytic flux and glycogen storage moieties in soleus and red and white gastrocnemius muscles (Fig. 2). This showed that [$^{14}$C]glucose incorporation into glycogen was somewhat increased in all three muscles but that the increase was significant only in red gastrocnemius of the fish group compared to the fat group. Net glycolytic flux accounted for most of the change in glucose metabolism in those tissues (soleus, 83%; red gastrocnemius, 68%; and white gastrocnemius, 75%), and in both soleus and red gastrocnemius there was a significant deterioration in the fat group and improvement in the fish group (Fig. 2).

No overall change in glucose metabolism in adipose tissue was seen in the fat group. In contrast to the effects in skeletal muscle, however, the fish group showed reduced insulin-stimulated glucose metabolism in white adipose tissue (Table 1). The shift in $R_g'$ from white adipose tissue to skeletal muscle is more clearly shown when calculated as a percentage change between the fat and fish groups (Fig. 3). Among the other tissues, heart and lung were unaffected in these two groups. In diaphragm and interscapular brown adipose tissue, $R_g'$ was lowered in the fat group, but this effect was not significantly reversed in the fish group (Table 1).

Thus replacement of only a small proportion of linoleic ω-6 fatty acids with long-chain polyunsaturated ω-3 fatty acids in a high-fat diet prevents the development of insulin resistance at the whole-body level in the rat. This improvement in insulin action is seen in the liver, the major organ of endogenous glucose production, and in skeletal muscle, the major site of glucose disposal. Comparing the two high-fat diets, the effect of fish feeding in skeletal muscle is primarily improved rates of insulin-mediated glucose oxidation or glycolysis, or both, but also includes improved rates of insulin-mediated glycogen storage.

The mechanisms of the effects of fish oils on insulin action are unclear. There are a number of chemical and structural differences between the fatty acids in safflower oil and fish oil. Compared to linoleic fatty acids (in safflower oil), eicosapentaenoic and do-

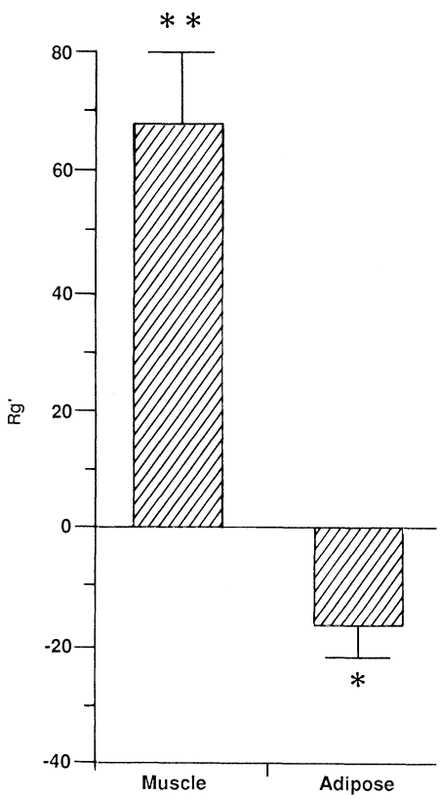


**Fig. 3.** Percentage change in $R_g'$ between the fat and fish groups ($n = 6$) in muscle (average of five hind-limb muscles) and white adipose tissue (average of three depots) in response to elevated concentrations of insulin. $*P < 0.05$, $**P < 0.01$.

cosahexaenoic fatty acids (in fish) are more unsaturated (two double bonds compared to five and six double bonds, respectively), of a longer chain length (18 carbons compared to 20 and 22 carbons, respectively), and of the ω-3 rather than ω-6 type. In addition to this last point, differences in metabolism of ω-6 and ω-3 fatty acids correlate well with altered production of various prostaglandins, thromboxanes, and prostacyclins (*18*), but the exact relation of these end-products to insulin action is unknown. It may be that the combination of ω-3 fatty acids and increased chain length is crucial. Studies with linolenic acid (an 18-carbon ω-3 fatty acid found, for instance, in linseed oil) would help to clarify this issue.

Another possible mechanism involves the altered membrane fluidity that results from the substitution of fish oil in the diet (*19*). Although more rigid membranes with the same number of receptors are capable of binding more insulin (*20*), events subsequent to binding, such as aggregation, internalization of the insulin-receptor complex, and movement of the glucose transporter to the cell membrane, could all be facilitated by changes in membrane fluidity.

Finally, ω-3 fatty acids are potent inhibitors of very low density lipoprotein (VLDL) synthesis in the liver (*21*). Since VLDL-triglycerides are an important energy source in peripheral tissues, a mechanism based on fuel switching with reduced fatty acid and increased glucose utilization (the glucose–fatty acid cycle of Randle) cannot be ignored.

Care must be taken when extrapolating results from rats to humans. The amount of fat in the high-fat diet (59% of the total calories) is greater than the average intake estimated for individuals in Western societies (40% to 45%). Further, the relation of fat intake to insulin resistance in humans has been established largely on the basis of epidemiological studies, and there is little evidence concerning alterations in insulin sensitivity in humans after the kind of changes in dietary fat described here. Finally, the substitution of ω-3 fatty acids in the rat diets, while small in terms of percentage, would still represent a large intake (8 to 9 g per day) in humans. Nevertheless, therapy combining modest increases in ω-3 fatty acid intake with general reduction in total fat may be particularly effective in the dietary treatment of non–insulin-dependent diabetes mellitus.


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15. The calculation assumes that the glucose metabolic index $R_g'$, derived from 2-DG phosphorylation, accurately estimates absolute glucose metabolic rate. There is evidence (*14*) that this assumption holds in skeletal muscle.
16. The values for $R_a$ and $R_d$ in Fig. 1, B and C, may be underestimates because of possible [$^{14}$C]glucose recycling (*13*). However, estimates based on plasma disappearance of isotopically labeled 2-DG (which overestimates $R_a$ and $R_d$ because of loss of tracer in urine) provide the same conclusion regarding relative diet effects. In absolute terms, if allowance is made for 15 to 20% recycling of labeled glucose at these insulin levels and for 16% loss of labeled 2-DG in urine (unpublished observations), then excellent agreement between the two calculations is achieved.
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# The *sor* Gene of HIV-1 Is Required for Efficient Virus Transmission in Vitro


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**The genome of the human immunodeficiency virus HIV-1 contains at least eight genes, of which three (*sor*, *R*, and 3′*orf*) have no known function. In this study, the role of the *sor* gene was examined by constructing a series of proviral genomes of HIV-1 that either lacked the coding sequences for *sor* or contained point mutations in *sor*. Analysis of four such mutants revealed that although each clone could generate morphologically normal virus particles upon transfection, the mutant viruses were limited in their capacity to establish stable infection. Virus derived from transfection of Cos-1 cells (OKT4$^-$) with *sor* mutant proviral DNA's was resistant to transmission to OKT4$^+$ "susceptible" cells under cell-free conditions, and was transmitted poorly by coculture. In contrast, virus derived from clones with an intact *sor* frame was readily propagated by either approach. Normal amounts of *gag*-, *env*-, and *pol*-derived proteins were produced by all four mutants and assays in both lymphoid and nonlymphoid cells indicated that their trans-activating capacity was intact and comparable with wild type. Thus the *sor* gene, although not absolutely required in HIV virion formation, influences virus transmission in vitro and is crucial in the efficient generation of infectious virus. The data also suggest that *sor* influences virus replication at a novel, post-translational stage and that its action is independent of the regulatory genes *tat* and *trs*.**


CONSIDERABLE PROGRESS HAS BEEN made in defining the genetic structure of HIV-1 and delineating the complex array of genes encoded by the 9.7-kilobase RNA molecule of this virus. To date, eight genes have been described: *gag, pol, env* (genes encoding conventional structural elements of the retrovirus), *tat*-III, *art*/*trs* (regulatory genes that are obligatory for virus replication), *sor, 3′orf*, and *R* (of undefined function) (*1*).

The *sor* gene (for short open reading frame) of HIV-1 (also called Q, P′, orf-1, and orf-A) lies between the *pol* and *tat* genes, overlapping at its 5′ end with the former (*2*). It is an open reading frame of 609 nucleotides in size and encodes a pro-


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