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# Adipocyte glucose transport regulation by eicosanoid precursors and inhibitors

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Iowa State University

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**Adipocyte glucose transport regulation by eicosanoid precursors  
and inhibitors**

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**Iowa State University, 1987**

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Adipocyte glucose transport regulation by  
eicosanoid precursors and inhibitors

by

He-Chong Cho Lee

A Dissertation Submitted to the  
Graduate Faculty in Partial Fulfillment of the  
Requirements for the Degree of  
DOCTOR OF PHILOSOPHY

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DEDICATION

TO MY PARENTS AND MY HUSBAND

## INTRODUCTION

Glucose is the principal monosaccharide released at the intestinal surface and accounts for 80% of digested carbohydrates. It is absorbed partly by diffusion and partly by Na-coupled,  $\text{Na}^+ - \text{K}^+$ -ATPase dependent active transport.

Among cells, brain, heart, and less importantly kidney have high dependencies on glucose for their energy. Adipose, muscle, and liver cells, on the other hand, utilize both glucose and fatty acids as their energy sources. Glucose transport into peripheral tissues is so important in energy metabolism that, if it is defective, there will be deficiencies of energy and abnormalities in energy utilization and storage in cells.

Glucose is transported into peripheral cells by facilitated diffusion which is catalyzed by an integral membrane protein, a transporter. By facilitated diffusion, glucose diffuses down in a glucose concentration gradient across cell membranes more rapidly than might be expected from its size, charge, or partition coefficients. The rate of diffusion can be saturated, and no metabolic energy is required even though the intracellular energy state could affect the overall rate of glucose transport.

The rate of glucose transport into peripheral cells is accelerated physiologically by several factors; hormones, particularly insulin; several multivalent membrane active agents including lectins, antimembrane antibodies, and antireceptor antibodies; agents that cause membrane perturbations including phospholipases and proteases (19).

Glucose is phosphorylated as rapidly as it penetrates. When insulin is not available in the system, the transport itself seems to be the rate-limiting step for the over-all uptake of glucose. When insulin is added, intracellular phosphorylation of glucose seems to be the rate limiting step for the over-all uptake of glucose (63, 67).

A high blood level of free fatty acids has been associated with an impaired glucose tolerance (79, 93) and decreased glucose utilization by peripheral tissues (80). On the contrary, a stimulatory effect of catecholamines on glucose transport has been reported (44, 45, 90). These conflicting results led to consideration of a factor which may be released into the incubation medium during stimulation of lipolysis and which mediates the effect of catecholamines in glucose transport.

Prostaglandins (PG), thromboxanes (TX), and prostacyclins (PGI) comprise groups of polyunsaturated, hydroxylated 20-carbon fatty acids, called collectively eicosanoids. They can evoke a wide spectrum of biological reactions, at extremely low concentrations, in a variety of tissues. Eicosanoids per se have been implicated as playing functional roles in such physiological processes as vasodilatation and vasoconstriction, regulation of body temperature, platelet aggregation, reproduction, inflammation, gastrointestinal functions, autonomic neuro-transmission, and cardiovascular and renal functions.

Eicosanoids are not stored within the cell, and it is widely believed that the rate limiting step in eicosanoid synthesis is precursor availability. Dietary manipulation of eicosanoid precursor

fatty acids can alter de novo synthesis of eicosanoids by altering the precursor availability at the cellular level (57). Relative amounts and kinds of dietary fat are directly and proportionally reflected in the composition of depot and erythrocyte lipids. Very high levels of precursor fatty acids in the diet may cause high levels of eicosanoids to be produced in cells.

Despite the myriad of biological and pharmacological properties of eicosanoids, relatively little is known of mechanisms by which these compounds act. This may be caused by their polyfunctional nature in a formidably wide spectrum of cellular effects. It is widely believed that the reaction site of eicosanoids is the membrane which houses specific sites for substrates similar to those of known hormone receptors and through which glucose is transported.

Many researchers have shown that eicosanoids are involved in carbohydrate metabolism and regulation of the blood level of glucose by three mechanisms; the modification of insulin release from the pancreas, the uptake and utilization of glucose by peripheral tissues, and glucose metabolism in liver. However, reports about eicosanoid effects on glucose metabolism are equivocal which may be attributed to experimental designs and executions. Effects of eicosanoids on peripheral glucose transport have not been extensively studied. Most of published reports suggest that eicosanoids, including  $PGE_1$ ,  $PGE_2$ , and  $TXA_2$ , but not  $PGA_1$  or  $PGI_2$ , may have stimulatory roles in peripheral glucose transport. Effects of eicosanoids on peripheral

glucose transport seem to be varied depending on types and relative amounts produced (53, 71, 91).

The overall objective of this research was to test the effects of endogenous eicosanoids on glucose transport into isolated fat cells using several modifiers of eicosanoid concentration. For this objective, rats were fed qualitatively different fats to serve as eicosanoid precursors, and fed aspirin to inhibit endogenous eicosanoid synthesis. Then ex vivo experiments were performed with isolated adipocytes harvested from rats fed experimental diets. Several in vitro treatments such as aspirin (a synthetic inhibitor of PG, TX, and prostacyclin production by an inhibition of cyclo-oxygenase activity), antiserum to PGE as a specific inhibitor of PGE, and norepinephrine (to stimulate release of eicosanoid precursors from fat cells) were also used to modulate eicosanoid concentration. Two-deoxy glucose uptake and free fatty acid release were measured during the incubation of adipocytes along with fatty acid composition in epididymal fat pads. Data of fatty acid composition of the fat pads, FFA released from the adipocytes, and eicosanoid levels in the adipocytes were treated statistically to find out relationships with glucose transport into adipocytes.



## REVIEW OF LITERATURE

## Glucose Transport

Glucose transporter

Glucose transport across cell membranes involves a specific interaction between glucose and a membrane component, glucose transporter, which has been found in a variety of tissues including rat adipocytes (38), human placenta, human skeletal muscle cells, chick embryo fibroblasts, and human erythrocytes (1). The transporter carries glucose alone, driven by its concentration gradient, and it is different from the  $\text{Na}^+$ -linked glucose transporter present in kidney and intestine. The transporter is a glycoprotein containing some 5%, by weight, sialic acid (86).

Structures and activities of the glucose transporter have been examined after isolating them from cell membranes and reconstituting them into artificial phospholipid vesicles (1). The data demonstrate that the reconstituted adipocyte glucose transporter has an apparent radius of about 60-80 Å indicating a region capable of spanning the plasma membrane. It has binding sites at both membrane sites, outward-facing and inward-facing. The reconstituted adipocyte glucose transporter also shows asymmetric kinetics for glucose uptake and efflux; the efflux has a four-fold greater maximum rate ( $V_{\text{max}}$ ) and four fold greater apparent affinity ( $K_{\text{max}}$ ) than the uptake processes (92). Using  $^3\text{H}$ -cytochalasin B, a reversible competitive inhibitor of glucose

transport, and an isoelectric focusing technique, Horuk et al. (38) found diverse glucose transporters different in isoelectric points (pH 6.4 and 5.6) in the low-density microsomes of rat adipocytes. In contrast, the plasma membrane glucose transporter was structurally homogeneous on isoelectric focusing, and only a single isoform focusing at pH 5.6 was detected. Horuk et al. suggested that pH 6.4 glucose transporter isoform might represent an immature species which was converted to the mature pH 5.6 form by differential glycosylation probably involving terminal capping with sialic acid residues. Chromatographic properties of adipocyte glucose transporters in detergent solutions are different from those of insulin receptors. This result indicates that the glucose transport proteins may be associated with insulin receptor proteins by only weak, noncovalent means that are disrupted by mild detergent actions (18).

The glucose transported into an extrahepatic cell is rapidly phosphorylated to glucose-6-phosphate by hexokinase activity; no free glucose accumulates inside the cell, and the rate of phosphorylation is limited by the rate of transport. In this state, the transport seems to be the rate-limiting step for the over-all uptake of glucose (16). When insulin is present, glucose transport is markedly stimulated; free glucose is found inside the cell. In this state, the accelerated transport by insulin exceeds the capacity of phosphorylation, which becomes the major limiting step for glucose transport (63).

### Factors affecting adipocyte glucose transport

Several agents with multivalent binding sites for the adipocyte plasma membrane such as lectins (concanavalin A and wheat germ agglutinin) and antibodies to plasma membranes or insulin receptors enhance glucose transport while decreasing lipolysis (20, 55, 68).

Czech and Lynn (19) found that the lectins, hemagglutinating substances that bind mannose-containing oligosaccharides, agglutinated the adipocyte membrane while increasing glucose transport. Even when the insulin binding to the brown adipocytes was completely blocked by trypsinization, lectin still enhanced 3-O-methylglucose transport compared with the control. Czech and Lynn suggested that the activity of lectin was mediated by affecting the D-glucose transporter, not by affecting insulin action.

Various antibodies also enhance D-glucose transport by adipocytes. Pillion and Czech (68) reported that antibodies raised in rabbits against adipocyte plasma membranes enhanced 3-O-methyl-glucose transport and CO<sub>2</sub> production while decreasing lipolysis either in trypsinized or in non-trypsinized adipocytes. Kahn et al. (47) reported that circulating antibodies against insulin in patients of acanthosis nigricans inhibited the binding of <sup>125</sup>I-insulin to adipocytes while activating glucose transport. These results demonstrate that membrane events elicited by multivalent agents which bind to cell surface sites other than insulin receptors activate glucose transport. On the other hand, monovalent F<sub>ab</sub> fragments of the

biologically active antibodies, prepared by papain digestion of the anti-membrane antibodies, were ineffective in enhancing glucose transport by adipocytes, suggesting that the ability of anti-membrane antibodies to enhance glucose transport by isolated adipocytes was dependent on the multivalent nature of the antibodies' structure.

Glucose concentration around the adipocyte modulates glucose transport. The rate of glucose transport increases when glucose concentration in the medium increases up to 5 mM. At higher than 5 mM glucose, the transport curve becomes linear suggesting a passive diffusion (93).

Phospholipase A has a biphasic effect on adipocyte glucose transport (3). At low concentrations such as lower than 3  $\mu\text{g}/\text{mL}$ , it stimulates glucose transport by adipocytes; at high concentrations such as higher than 3  $\mu\text{g}/\text{mL}$ , it inhibits glucose transport. Similar, concentration-related, biphasic effect of phospholipase C on adipocyte glucose transport has been also reported (75).

Microviscosity of the membrane may affect glucose transport by adipocytes. Melchior and Czech (59) observed that the glucose transport activity in a reconstituted, membrane protein containing phospholipid vesicle containing adipocyte membrane protein was parallel to the increment of temperature from 0°C to 30°C at which the bilayer fluidity was maximal. Above 30°C, the transport activity was increased less markedly. Furthermore, when the reconstituted membrane vesicle was prepared with cholesterol, the microviscosity was increased while inhibiting glucose transport at 23°C.

The oxido-reductive state may affect glucose transport. Ludvigsen and Jarett (55) reported that oxidants such as  $H_2O_2$  and diamide enhance, while reductants inhibit glucose transport by adipocytes.

Insulin, a hormone produced by the beta cells of the islets of Langerhans in the pancreas and secreted into the blood as a direct response to hyperglycemia, plays a central role in the regulation of glucose transport by extrahepatic cells including adipocytes. The ability of insulin to stimulate glucose metabolism in adipose tissue was first observed by Winegard and Renold in 1958 (95). Later it was found that insulin caused a manyfold increase in the  $V_{max}$  without altering the  $K_{max}$  of glucose transport activity, suggesting that the stimulatory effect occurred at the glucose transport level (55, 93).

In vitro studies with isolated rat adipocytes show that the onset of insulin's action takes up to 45 seconds at  $37^\circ C$  (83). The first step in the transfer of the messages of insulin into the cell is an interaction of insulin with its specific receptors on the membrane. An insulin receptor appears to be an integral membrane protein with a radius of 68-72  $\text{A}^\circ$ , comprised of 3-4 subunits, and it has an estimated molecular weight of 300,000 to 1,000,000 Daltons. The half life of an insulin receptor is about 16 min; it shows characteristics such as saturability, reversibility, specificity for substrates, pH dependency, temperature dependency, ion sensitivity, and negative cooperativity (46).

Morphologic studies show that insulin localizes to the plasma membrane producing a receptor-insulin complex, aggregates the membrane sites, and then the receptor-insulin complex is rapidly internalized into the cell. The endocytosed complex may be metabolized in the lysosome or recycled back to the plasma membrane (39)

Only a small fraction of existing insulin receptor sites on the membrane have to be occupied to exert the maximal effect of insulin on glucose transport. Simpson and Cushman (83) reported that 0.1 to 1.0 nM insulin caused 5 to 95 per cent of the stimulatory effect on 3-O-methyl glucose transport into rat adipose cells. A half maximal stimulation was caused with 0.3 nM which corresponded to a receptor occupancy of about 10 per cent. This discrepancy between the cell's binding capacity for insulin and the amount of insulin required for the maximal action has given rise to the concept of spare receptors.

On the other hand, the concentration of circulating insulin inversely regulates the number of insulin receptors (down regulation). This down regulation may be a factor of insulin resistance in which the insulin effect at the target cell is diminished.

The mechanism by which insulin stimulates glucose transport is not clear yet. Recent work strongly suggests that insulin may increase the number of operational glucose transporters by translocating them from a non-operative, intracellular pool or by modifying the existing non-operative transporters (39, 41, 83). These concepts are in accordance with earlier data that insulin enhances the  $V_{\max}$  without altering the

$K_{max}$  of the glucose transport curve (55, 93). However, little is known about the signals elicited by receptor binding of insulin and the activation of the glucose transporter.

An increased concentration of plasma free fatty acids is associated with an impaired glucose tolerance as observed in patients with endocrine and metabolic disorders such as genetic diabetes mellitus, hormone induced diabetes, prolonged starvation, carbohydrate deprivation, and familial hyperlipoproteinemia (79, 93). Stimulation of lipolysis by agents increasing the activity of adenylate cyclase reduced binding and the effects of insulin on glucose transport (10). Moreover, short, medium, and long chain fatty acids, as well as their metabolites such as acetoacetate and  $\beta$ -hydroxybutyrate, inhibit peripheral glucose transport and utilization (80, 82).

On the contrary, a stimulatory effect of catecholamines on glucose transport has been reported. First, Vaughan in 1961 (90) reported that lipolytic hormones, norepinephrine at 2.7  $\mu\text{g/mL}$ , and epinephrine at 2.7  $\mu\text{g/mL}$  caused a 1.3- to 3-times increase in glucose transport by adipose tissue compared with the control values. Recently, many researchers have reported that  $\beta$ -adrenoceptor agonists such as L-isoproterenol enhanced glucose transport by adipocytes. This enhancement was closely parallel to the stimulation of lipolysis and was abolished by propranolol, a beta adrenoceptor blocker (44, 45).

In white adipocytes from hamster and human adipocyte tissue, it is possible to see  $\beta_1$ ,  $\beta_2$ ,  $\alpha_1$ , and  $\alpha_2$  effects separately,

based on structure-activity relationships for agonists (26). Activation of beta adrenoceptor stimulates, while activation of alpha<sub>2</sub> adrenoceptor inhibits cyclic AMP and lipolysis. The cyclic AMP and lipolytic response to catecholamines may depend on the relative ratio of alpha<sub>2</sub> to beta effects. Alpha<sub>1</sub> adrenoceptors do not have known effects on lipolysis, rather their activities are associated with an elevated concentration of cytosolic calcium or with an elevated turnover rate of phosphatidyl inositol. It also stimulates glycogen-phosphorylase activity and inhibits glycogen synthase activity via mechanisms distinct from those effects of adenylate cyclase activation. Norepinephrine is more potent than epinephrine in activating lipolysis. Norepinephrine released from sympathetic nerves may activate beta<sub>1</sub> adrenoceptor to increase concentration of cyclic AMP and lipolysis. Epinephrine released from the adrenal medulla may activate beta<sub>2</sub> adrenoceptor to increase cyclic AMP concentration and dilate blood capillaries supporting adipose tissue (26).

#### Usage of nonmetabolizable glucose analogs

The radiolabeled glucose analog, 2-deoxy-D-glucose (2-DG), is transported into adipocytes and phosphorylated by hexokinase by the same process as D-glucose does. It cannot be further metabolized into CO<sub>2</sub> or transformed into lipids and is trapped intracellularly. Theoretically, uptake represents the balance between influx and efflux. To assess initial transport rates, it is essential to measure influx under circumstances where efflux is minimal or non-existent. The



measurement of 2-DG transport provides an accurate assessment of glucose transport provided that the rate of phosphorylation by hexokinase is rapid enough to prevent any accumulation of non-phosphorylated deoxyglucose, e.g., the transport itself is rate-limiting.

3-O-Methyl-D-glucose (3-OMG) is another glucose analog widely used to study glucose transport. However, unlike 2-DG, 3-OMG is not phosphorylated, and consequently, it is not trapped within the cell. Since the adipocytes' intracellular water space is comparatively small (2-4 per cent of the cell volume), the intracellular concentration of this sugar rapidly rises, leading to significant efflux at very early time points. Thus the initial rates of glucose transport must be measured within a few seconds (27). Shortening of the assay time may result in a considerable decrease in the sensitivity of the assay because of the problems of mixing the label with the cells and of stopping the 3-OMG transport without efflux of label. Olefsky (64) reported that 3-OMG transport kinetics showed a non-saturable process and might not completely reflect the D-glucose transport system. Furthermore, phloretin and phlorizin, which are often used in stopping 3-OMG transport by adipocytes, have been reported to cause drastic effects on fat cell metabolism (22, 93).

## Eicosanoid Metabolism

The nutritional requirement of mammalian species for certain fatty acids was first recognized in young rats and described by Burr and Burr (6, 7). These investigators demonstrated that rats, maintained on a fat-free diet over a long period, developed an abnormality characterized by scaliness of the dorsal skin, the feet, and the tail. This abnormal skin later became necrotic. These signs were accompanied by growth retardation, impaired fertility, increased water consumption and diminished urine production. Burr and Burr proposed that linoleic and possibly linolenic acids were essential fatty acids. On the other hand, arachidonic acid has been considered to be the principal unsaturated fatty acid required by the animal organism (84). This line of thought has been strengthened by reports from several investigators who have shown that linoleic and  $\gamma$ -linolenic acids undergo transformations in the animal organism to give arachidonic acid (70). Later studies have demonstrated that essential fatty acids can be transformed enzymatically into eicosanoids which act as local hormones and evoke a wide spectrum of biological reactions at extremely low concentrations in a variety of tissues.

### Synthesis of eicosanoids

Linoleic acid is the most important dietary precursor of eicosanoids. It undergoes a single desaturation and a elongation step to form dihomo- $\gamma$ -linolenic acid which is the precursor of group one

(mono-ene) prostaglandins, thromboxanes, and prostacyclins (these three groups of compounds are referred to herein as PG). Further desaturation produces arachidonic acid which is the precursor of group two (di-ene) PG. Alpha-linolenic acid is another dietary precursor of PG. It undergoes desaturation and elongation steps to produce eicosapentaenoic acid which is the precursor of group three (tri-ene) PG. Group two PG are physiologically the most important ones.

Most cellular arachidonic acid is found in the membrane phospholipid fraction, and its release is facilitated by membrane bound phospholipase activities such as phospholipase A<sub>2</sub> activity or phospholipase C activity followed by 1,2-diacyl glycerol hydrolase activity. Arachidonic acid is usually attached to the carbon two position of the phospholipid.

Arachidonic acid is metabolized by two distinct pathways known as the arachidonic acid cascade producing the family of compounds called eicosanoids. Arachidonic acid is hydroxylated in one pathway by a cytosolic enzyme, fatty acid lipoxygenase, and produces unstable peroxygenated fatty acids such as hydroperoxy eicosatetraenoic acid (HPETE) which is reduced to hydroxy eicosatetraenoic acid (HETE). Peroxidation at C-5 also leads to the synthesis of a family of leucotrienes. Alternatively, arachidonic acid interacts with a membrane bound multienzyme complex, prostaglandin endoperoxide synthetase. This enzyme complex has two enzyme activities, fatty acid cyclo-oxygenase and prostaglandin hydroperoxidase activities. It

specifically catalyzes the incorporation of molecular oxygen into polyunsaturated fatty acids, generating 15-hydroxy prostaglandin endoperoxides (PGG<sub>2</sub> and PGH<sub>2</sub>). Prostaglandin endoperoxides are the common precursors of group two PG (56). Prostaglandin endoperoxide synthetase seems to be present in all cell types and present intracellularly in the microsomal fraction (34).

PGG<sub>2</sub> and PGH<sub>2</sub> are also biologically active compounds that induce platelet aggregation and mediate pressor effects on vascular smooth muscle. They are unstable (half-life approximately 5 min in smooth muscle at 37°C in aqueous solution) and are quickly isomerized to specific PG by specific synthetic enzymes (31, 32).

Transformation of PGH<sub>2</sub> to PGD<sub>2</sub> is catalyzed by PGD<sub>2</sub> isomerase, and transformation of PGH<sub>2</sub> to PGE<sub>2</sub> is catalyzed by PGE<sub>2</sub> isomerase. PGD<sub>2</sub> isomerase and PGE<sub>2</sub> isomerase are probably glutathione dependent enzymes. PGF<sub>2α</sub> is transformed from PGH<sub>2</sub> by a non-enzymic process or from PGE<sub>2</sub> by 9-keto-reductase (14, 96).

The prostaglandin endoperoxides can also be transformed enzymatically into two other unstable products with potent biological activities called thromboxane A<sub>2</sub> (TXA<sub>2</sub>) and prostacyclin (PGI<sub>2</sub>). TXA<sub>2</sub> is the major product of arachidonic acid metabolism in platelets and prostacyclin is the major product of arachidonic acid metabolism in vascular tissues. TXA<sub>2</sub> is a potent vasoconstrictor and inducer of platelet aggregation, while PGI<sub>2</sub> is a powerful vasodilator and inhibitor of platelet aggregation. The balance of these two activities

appears to be important in cardiovascular functions (88).  $\text{TXA}_2$  is transformed from  $\text{PGH}_2$  by thromboxane synthetase. The compound is very short-lived (half-life of about 30 s in vitro and is quickly hydrolyzed to stable, but biologically inactive  $\text{TXB}_2$  (33).  $\text{PGI}_2$  is transformed from  $\text{PGH}_2$  by prostacyclin synthetase. This compound is also short-lived (half-life of about 2-3 min at  $37^\circ\text{C}$  in blood) and is quickly hydrolyzed to stable 6-keto- $\text{PGF}_{1\alpha}$  (62). In addition,  $\text{PGG}_2$  and  $\text{PGH}_2$  are also precursors of a 3-carbon substance, malondialdehyde (MDA), and a 17-carbon hydroxy acid, 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT) (88).

Eicosanoids are not stored in mammalian tissues, and various physiological or physical stimuli cause rapid biosynthesis of eicosanoids. It is generally considered that the availability of free arachidonic acid is the rate limiting factor in the formation of eicosanoids in vivo. The biosynthesis of eicosanoids is shown in Figure 1.

Fatty acid composition of tissue lipids is affected by the fatty acid composition of dietary fats. Kinds of dietary fats can affect the availability of eicosanoid precursor acids and the synthesis of eicosanoids by altering the composition of endogenous precursor fatty acid pool (57). Evidence for this synthetic difference was demonstrated by reduced synthesis of eicosanoids in essential fatty acid deficient (EFAD) rats (13), elevated synthesis of eicosanoids in the high linoleic acid-fed rat (40, 57), and cleared dermal symptoms of

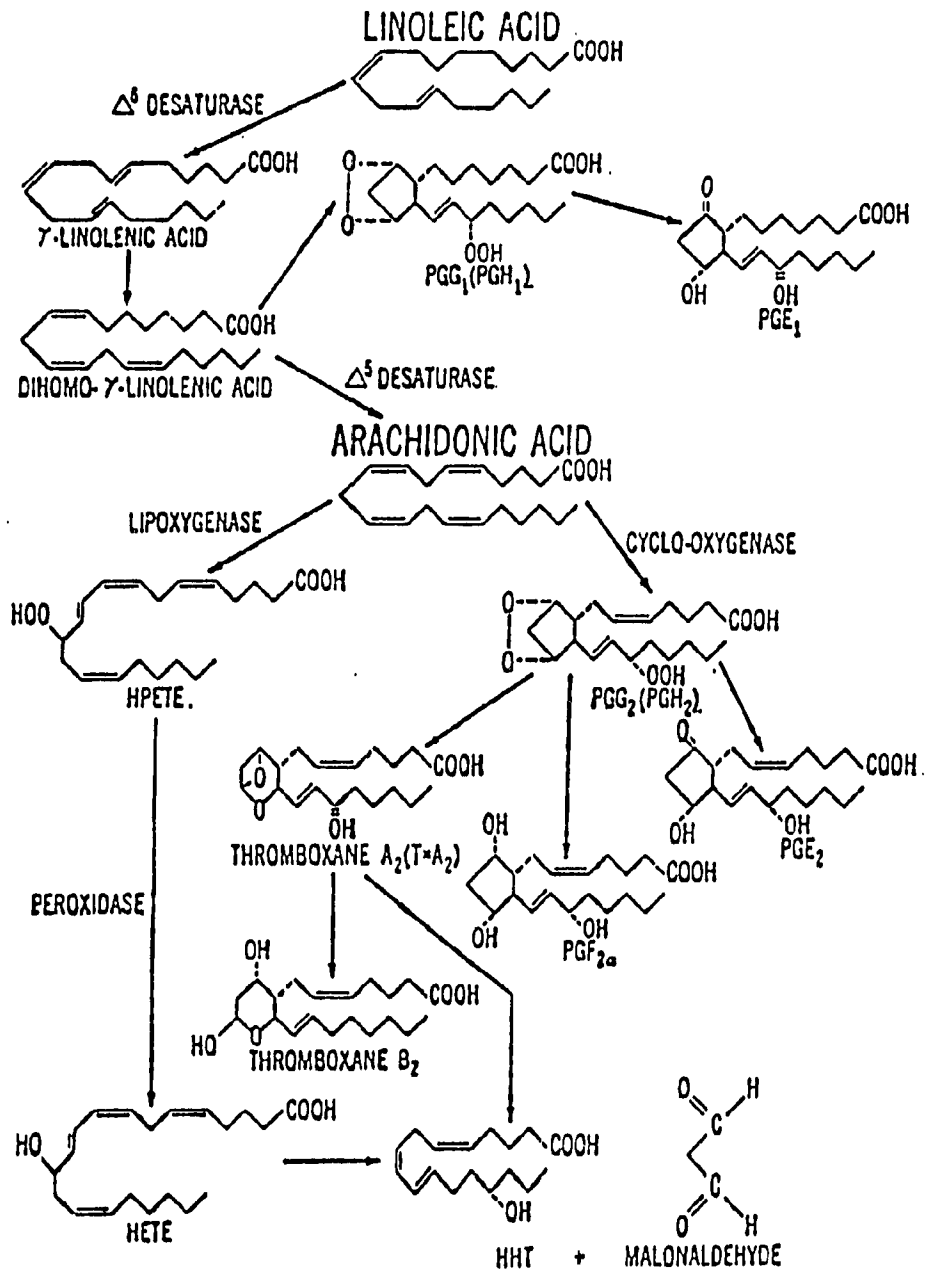


FIGURE 1. Pathways of incorporation of linoleic acid into eicosanoids

EFAD rats by topical application of PGE<sub>2</sub> (98). In a review article, Mathias and Dupont (57) concluded that synthesis of eicosanoids can be depressed by biochemical essential fatty acid deficiency and enhanced when the linoleic acid to saturated fatty acid ratio (P/S) of the dietary fat was greater than 5.

The trans-isomers of fatty acids produced during partial hydrogenation of fats have been reported to decrease synthesis of eicosanoids (9). And the presence of exogenous oleic, linoleic, elaidic, vaccenic, and  $\alpha$ -linolenic acids were also inhibitory to the conversion of linoleic acid to  $\gamma$ -linolenic acid or to the conversion of <sup>3</sup>H-arachidonic acid to PGE<sub>2</sub> in sheep vascular glands or in rat stomach in vitro. Alpha-linolenic acid caused the greatest inhibition (66). The inhibition probably was caused by a competition among fatty acids for desaturases or for prostaglandin synthetase (4, 37).

#### Catabolism of eicosanoids

Major sites of catabolism of eicosanoids are lungs, kidneys, intestines, liver, and placenta. However most eicosanoids seem to be catabolized at a place near to their synthesis (88). The main enzymes responsible for the biological inactivation of eicosanoids by converting the 15-hydroxyl group into the keto form are generally termed 15-hydroxy-prostaglandin dehydrogenases (15-OH-PGDH). These enzymes are generally present in mammalian tissues. Following the initial oxidation of the 15-hydroxyl group; 15-keto prostaglandins, thus obtained, undergo a NADH or NADPH dependent irreversible reaction

between C-13 and C-14 to form 15-keto 13,14-dihydro prostaglandins. The 15-keto 13,14-dihydro prostaglandins appear to be the initial major metabolites in the plasma. The enzyme responsible for this reduction is 15-keto prostaglandin  $\Delta^{13}$ -reductase which is also present in most tissues.

Fifteen-keto 13,14-dihydro prostaglandins are further catabolized by two cycles of the  $\beta$ -oxidation of the carboxyl side chain and by  $\omega$ -oxidation resulting in the excretion of C-16 dicarboxylic acids in the urine (88).

#### Effects of aspirin on the synthesis of eicosanoids

The pioneer studies by Vane (89) showed that non-steroidal anti-inflammatory aspirin (acetylsalicylic acid) and indomethacin inhibited biosynthesis of PG from arachidonic acid by cell-free homogenates of guinea-pig lung. These reports stimulated the search and the evaluation of a variety of non-steroidal, anti-inflammatory drugs such as meclofenamic acid, indomethacin, mefenamic acid, flufenamic acid, maproxen, phenylbutazone, aspirin, and ibuprofen. The precise mechanism of the inhibitory action is still uncertain; however, these drugs block the initial stage of the prostaglandin endoperoxide synthetase either in a reversible or in an irreversible manner. Irreversible inhibitors can cause prolonged reduction of synthetic activity, and presumably, synthesis of new cyclo-oxygenase is necessary to produce PG. The reversible inhibitors such as indomethacin prevent the formation of PG only as long as the agents are present in a



sufficient concentration to compete with the existing pool of free substrates. Aspirin (acetylsalicylic acid) is an irreversible inhibitor, and it selectively acetylates the fatty acid cyclooxygenase; 8-10 days are required for platelets treated with this drug to recover and to synthesize PG (76).

#### Effects of Eicosanoids on Glucose Metabolism

According to reports, eicosanoids influence glucose metabolism in several aspects: pancreatic insulin release, hepatic glucose metabolism, and peripheral glucose transport and metabolism.

#### Effects of eicosanoids on insulin release

Bressler et al. in 1968 (5) reported that PGE<sub>1</sub> at 2.5 to 5 µg injection increased both plasma insulin and blood glucose in mice in vivo. Since then many investigators have studied the effects of eicosanoids on beta-cell function in a variety of investigative models. These models have ranged from monolayer cultures of islet cells to human investigation.

In vitro experiments Johnson et al. (42) reported that PGE<sub>1</sub>, PGE<sub>2</sub>, and PGF<sub>2α</sub>, but not PGA<sub>1</sub>, enhanced release of insulin in isolated pancreatic islets which were incubated with high glucose media (300 mg%). The effect of eicosanoids was dose related. PGE<sub>1</sub>, the effect of which was apparent at 10<sup>-8</sup> M, was the most potent in stimulation of insulin release. However, even PGF<sub>2α</sub> and PGE<sub>2</sub> caused nearly a twofold

increase in glucose-stimulated insulin release at  $10^{-4}$  M concentration. Burr and Sharp (8) also reported a stimulatory effect of  $\text{PGE}_1$  (0.3  $\mu\text{g/mL}$ ) on insulin release from perfused, isolated pancreatic islets of rats. In that experiment,  $\text{PGE}_1$  was effective only within media containing low glucose concentration (50 mg%); in the media containing high glucose concentration (300 mg), it rather inhibited the insulin release.

Landgraf and Landgraf-Leurs (50) reported that  $\text{PGF}_{2a}$  affected insulin release from isolated, perfused rat pancreas, but  $\text{PGE}_2$  did not. The effect of  $\text{PGF}_{2a}$  was dose related; a low exogenous  $\text{PGF}_{2a}$  (1  $\mu\text{M}$ ) was stimulatory while high  $\text{PGF}_{2a}$  (10  $\mu\text{M}$ ) was inhibitory. The total effects of endogenous eicosanoids were stimulatory in these experiments; synthetic inhibitors of eicosanoids (indomethacin<sup>1</sup> or mepacrine<sup>2</sup>) inhibited insulin release, while a treatment of catabolic inhibitor of eicosanoids (furosemide)<sup>3</sup> stimulated it.

Most in vitro studies showed that eicosanoids affect pancreatic insulin release in a positive manner. This effect of eicosanoids on insulin release may be closely related to the activation of the adenylate cyclase system. In fact, eicosanoids increase cyclic AMP in islets of Langerhans by increasing the activity of adenylate cyclase (50, 94). Glucose concentrations in the media and/or eicosanoid types

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<sup>1</sup>An inhibitor of PG endoperoxidase.

<sup>2</sup>An inhibitor of phospholipase.

<sup>3</sup>An inhibitor of PG dehydrogenase.

and concentrations may influence the effect of eicosanoids on insulin release.

Experiments in intact animals Lefebvre and Luyskx (52) reported that PGE<sub>1</sub> infusion at the i.v rate of 1 µg/Kg/min for 40 min significantly stimulated insulin output in overnight fasted, anesthetized dogs, while a lower dose (0.5 µg/Kg/min) did not affect insulin output. Later, Schusdziarra et al. (81) found that the stimulatory effect of PGE<sub>2</sub> on insulin release was transitory. When they infused PGE<sub>2</sub> i.v. at 1 µg/Kg/min into anesthetized dogs for 50 min, the insulin level in the pancreatic vein was gradually increased during the first 25 min and gradually decreased to the basal level during the later 25 min. In this study, PGE<sub>2</sub> caused hyperglycemia even during the period of the elevated insulin level in blood.

On the other hand, Sacca et al. (77) reported a lowering effect of exogenous PGE<sub>1</sub>, PGE<sub>2</sub>, and PGA<sub>1</sub> at 2 µg/min on plasma insulin level. Robertson (72) also reported that infusion of PGE<sub>1</sub> and PGE<sub>2</sub> i.v. at 10 µg/min in dogs decreased basal or glucose-induced insulin release.

Experiments in humans Robertson and Chen (73) reported that the infusion of PGE<sub>2</sub> at 10 µg/min inhibited acute insulin response to a glucose pulse (i.v. 5 g) in normal people. Sodium salicylate, an inhibitor of endogenous eicosanoid synthesis, augmented acute insulin response to glucose pulse in normal people. Furthermore, in noninsulin-dependent diabetes (NIDDM) with fasting hyperglycemia,

sodiumsalicylate restored acute insulin response to glucose pulse. This restoration of acute insulin response to glucose pulse was accompanied by a four-fold augmentation in second phase insulin release. Giugliano et al. (29) reported that sodium salicylate infusion at 40 mg/min for 60 min caused a decrease in blood insulin levels in normal, healthy subjects; it did not affect blood insulin level in insulin-dependent diabetic patients.

Ray et al. (69) reported a direct role of PGE<sub>1</sub> and prostacyclin in the biological effect of insulin in vitro. They showed that PGE<sub>1</sub> or prostacyclin at 3 nM increased the binding of <sup>125</sup>I-labeled insulin to the human erythrocyte membrane preparation. Higher concentrations (above 8 nM) of the eicosanoid tended to reverse its effect on increasing insulin binding. A Scatchard analysis of the insulin binding to the erythrocyte membrane indicated that PGE<sub>1</sub> increased the binding capacity ( $V_{max}$ ) 2 times, without changing  $K_{max}$ . PGE<sub>2</sub>, either at 3 nM or 8 nM, decreased insulin binding, and other eicosanoids (PGA<sub>1</sub>, PGA<sub>2</sub>, PGB<sub>1</sub>, PGB<sub>2</sub>, PGD<sub>2</sub>, and PGF<sub>2a</sub>) had no effect.

On the other hand, Szczeklik et al. (87) could not find any apparent effect of PGI<sub>2</sub> infusion (i.v. 5 ng/Kg/min) of 72 h into non-diabetic, cardiovascular disease patients on glucose-stimulated insulin response. Patients showed increased, or decreased, or non-affected results.

Conclusions      Studies have revealed conflicting data about the role of eicosanoids on insulin release; stimulatory as well as

inhibitory functions of various eicosanoids were observed. Considering the complex behavior of eicosanoids, the conflicting results are not surprising. However, the following factors should be considered in the study of eicosanoids effects on insulin release.

1. The level of administration of eicosanoids through i.v. may not reflect their levels at the pancreatic cells, since eicosanoids are very quickly metabolized and normally do not circulate through the systemic blood system.
2. Most studies used pharmacological rather than physiologic concentrations of exogenous eicosanoids which may exert diverse pharmacological effects in the body, and these may affect the pancreatic  $\beta$ -cells function indirectly.
3. The concentration of glucose in the area surrounding the pancreas may affect the role of eicosanoids in insulin release.

Reported studies revealed significant effects of eicosanoids on pancreatic insulin release. Further studies are needed to confirm the role of eicosanoids on insulin release.

#### Effects of eicosanoids on hepatic glucose metabolism

Usually PGE<sub>1</sub>, PGE<sub>2</sub>, and PGI<sub>2</sub> cause hyperglycemia when administered exogenously (78, 81). This hyperglycemic effect of eicosanoids could be explained by: increased glucose production of liver, decreased

glucose utilization of peripheral tissues, or both. Several investigators have reported that eicosanoids influence carbohydrate metabolism at the hepatic level (17, 78). Curnow and Nuttal (17) found that PGE<sub>1</sub> stimulated phosphorylase activity and decreased glycogen synthetase activity in isolated, perfused rat liver.

Sacca et al. (78) reported that i.v. infusion, for 60-75 min, of 2 µg/min of PGE<sub>1</sub> and PGE<sub>2</sub> into rats fasted for 18-20 h caused significant hyperglycemia. The rate of glucose turnover was calculated using the formula of Steele<sup>4</sup>, and the result showed that the hyperglycemia was due to an enhanced glucose production by liver. PGE<sub>1</sub> showed a more profound effect than PGE<sub>2</sub> in causing hyperglycemia. The glucose uptake by peripheral tissues was also calculated; glucose uptake was immediately increased when the infusion of PGE<sub>1</sub> or PGE<sub>2</sub> was started, and kept increasing to reach the level close to that of hepatic output at the end of infusion. This finding suggests that PGE<sub>1</sub> and PGE<sub>2</sub> have regulatory roles on the level of blood glucose by stimulating glucose production by liver and glucose utilization by peripheral tissues.

Since PGE<sub>1</sub>, PGE<sub>2</sub>, and PGI<sub>2</sub> infusion cause modest hypotension, reflexly released catecholamine might secondarily cause the hyperglycemia. However, this possibility seems to be ruled out. PGI<sub>2</sub> infusion, at 5 ng/Kg/min into cardiovascular diseased or at 0.1-1.0

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<sup>4</sup>Steele's formula, after administering i.v. labeled glucose, calculates glucose turnover rate in the body by measuring the infusion rate, appearance rate in the blood, and the disappearance rate in the blood of the labeled-glucose.

$\mu\text{g}/\text{Kg}/\text{min}$  into healthy rabbits for 3 h, caused hyperglycemia in nearly all animals while causing hypotension in only 20% of them. These results suggest that the hyperglycemic effect of eicosanoids was not caused by reflexly released catecholamines. Furthermore, inhibitions of catecholamines by a depletion of catecholamine stores by reserpine or by administering an  $\alpha$ -adrenergic blocker, phentolamine, did not change the hyperglycemic effect induced by eicosanoids (72).

Hyperglycemia is a common feature in diabetes. And the production of  $\text{PGE}_1$ ,  $\text{PGE}_2$ , and  $\text{PGF}_{2\alpha}$  in the serum is increased in diabetic patients (43, 12). These elevated eicosanoids might be contributing factors to hyperglycemia by increasing hepatic glucose production in diabetes.

#### Effects of eicosanoids on peripheral glucose transport

Several papers have reported that eicosanoids have insulin-like effects on glucose metabolism in fat cells. Vaughan (91) demonstrated an increase in glucose- $\text{U-}^{14}\text{C}$  uptake and its conversion into glycogen and fatty acids by exogenous  $\text{PGE}_1$  (0.4 or 1.0  $\mu\text{g}/\text{mL}$ ) in rat adipose tissue in vitro. Later, several other researchers (11, 28, 97) also reported a similar, stimulatory effect of  $\text{PGE}_1$  on glucose transport by adipose tissue or into isolated adipocytes, in vitro.

Richelson et al. (71) reported that exogenous  $\text{PGE}_2$  stimulated 3-O-methyl glucose transport into isolated human subcutaneous fat cells. ( $\text{U-}^{14}\text{C}$ )Glucose oxidation into  $\text{CO}_2$  and lipogenesis were also stimulated by  $\text{PGE}_2$ . The effect of  $\text{PGE}_2$  was dose dependent and manifested as low as 0.8 nM concentration. In this study, indomethacin significantly

inhibited insulin-stimulated  $\text{CO}_2$  production in fat cells, whereas it had no effect on basal glucose metabolism. Exogenous  $\text{PGF}_{2\alpha}$  tended to increase 3-O-methyl glucose transport which was not statistically significant. Lefebvre and Luyskx (53) reported that L8027 (pyridyl indolyl ketone, a specific inhibitor of  $\text{TXA}_2$  synthesis) decreased glucose transport by rat adipose tissue, suggesting a stimulatory effect of endogenous  $\text{TXA}_2$  on glucose transport.

Begum et al. (2) also reported that endogenous eicosanoids stimulate intracellular glucose metabolism. Indomethacin treatment at 3  $\mu\text{g}/\text{mL}$  to the isolated adipocytes or particulate fraction containing mitochondria and plasma membrane of rat liver inhibited pyruvate dehydrogenase activity by approximately 62% as measured by  $^{14}\text{CO}_2$  production from 1- $^{14}\text{C}$ -pyruvate.  $\text{PGE}_2$  addition at  $1.2 \times 10^{-6}$  M into the incubation system completely overcame the inhibitory effect of indomethacin. Dexamethasone, a glucocorticoid analog and an inhibitor of eicosanoid synthesis, at 1  $\mu\text{M}$  also decreased insulin activation of pyruvate dehydrogenase activity which was partially restored by addition of arachidonic acid (0.2  $\mu\text{g}/\text{mL}$ ).

$\text{PGE}_1$  and  $\text{PGE}_2$  also stimulated glucose transport and metabolism in myometrium from rhesus monkeys (15), isolated stripped soleus muscle of rats (54), and canine thyroid tissue (65). But  $\text{PGE}_2$  and  $\text{F}_{2\alpha}$  suppressed (U- $^{14}\text{C}$ )-glucose oxidation into  $^{14}\text{CO}_2$  in cultured mouse embryo (49).

Szczeklik et al. (87) reported that the effect of  $\text{PGI}_2$  on peripheral glucose uptake was different between diabetic and non-



diabetic subjects. Five to ten ng of i.v. PGI<sub>2</sub>, administered to non-diabetic but peripheral vascular disease patients, did not show a significant effect on glucose uptake in vivo. But an equivalent amount of PGI<sub>2</sub>, administered to diabetic patients, caused a decrease in glucose uptake by peripheral tissue.

Summary Effects of eicosanoids on peripheral glucose transport have not been extensively studied. Most of the published reports are suggesting that eicosanoids, including PGE<sub>1</sub>, PGE<sub>2</sub>, and TXB<sub>2</sub>, but not PGA<sub>1</sub> or PGI<sub>2</sub>, may have stimulatory roles in glucose transport by adipocytes. Effects of eicosanoids on peripheral glucose transport seem to be varying depending on types and relative amounts of eicosanoids produced.

#### Hypothesis

Eicosanoids are involved in glucose transport into adipocytes.  
The quantity of dietary C18:2 affects eicosanoids and may affect glucose transport.

## EXPERIMENTAL DESIGN

One-month-old Zucker rats (twenty rats per experiment, 10 rats per diet, 5 rats per diet group) were fed iso-nutrient diets high (HSO) or low (LSO) in safflower oil for one month. After one month, five rats per diet group were fed aspirin for two days at 50 mg/Kg bw/day (ASP group), and the remained 5 rats in the same diet group were continued on the previous diets (HSO or LSO) without aspirin (CON group). At the end of one month feeding, there were four diet groups (HSO-CON, HSO-ASP, LSO-CON, LSO-ASP). Experiments were repeated four times.

TABLE 1. Experimental design

In vivo		In vitro
Diet	Group	Adipocyte Treatment
High 18:2 (HSO)	+ Asp (ASP)	<u>+</u> Norepinephrine (N)
	- Asp (CON)	<u>+</u> Aspirin (Asp) <u>+</u> Anti-PGE (AntiE)
Low 18:2 (LSO)	+ Asp (ASP)	<u>+</u> Asp x Norepi (AspN)
	- Asp (ASP)	<u>+</u> AntiE x N (AntiEN)

Epididymal fat pads from 5 rats in each diet group were removed and pooled. Part of the fat pad was used to analyze fatty acid composition. Part of it was used for isolating adipocytes and performing in vitro glucose transport studies. The isolated adipocytes were distributed into 8 flasks and incubated with eicosanoid modifiers (norepinephrine, N; aspirin, Asp; antiserum to PGE, AntiE; and combinations of Asp x N, or AntiE x N) for 15 min. During the incubation, 2-DG transport and free fatty acid release were measured. Data were analyzed statistically to test the effect of diets on the composition of fatty acids of epididymal fat pads, the effect of the composition of fatty acids of fat pads on the composition of fatty acids released from isolated adipocytes, the effects of diets on the glucose transport into isolated adipocytes, and the effects of fatty acids released from isolated adipocytes on the glucose transport into adipocytes. The statistical methods used were Analysis of Variance, General Linear Models Procedure, and Least Significant Difference, at a 5% levels of probability. Pearson Correlation Coefficients were also determined and were deemed significant if the  $r \geq 0.5$  and  $P \leq 0.05$ .

## METHODS AND MATERIALS

## Animals and Care

In all the studies, non-obese, male Zucker rats raised in the stock colony of Iowa State University, Food and Nutrition Laboratory were used. One-month-old rats were individually caged in stainless steel cages, subjected to a 12 h light-dark cycle (0600-1800h light), and maintained at 24°C and 50% relative humidity. Twenty rats per experiment were evenly distributed into two experimental diet groups to give the same mean initial body weights between dietary groups. Rats were fed test diets which were different only in lipid qualities. High safflower oil diet (HSO) contained 20% of total calories from safflower oil as the only fat source. Low safflower oil diet (LSO) contained 2% of total calories from safflower oil and 18% from beef tallow (Table 2).

At the end of 4 weeks feeding, 5 rats per diet group were fed aspirin at 50 mg/Kg bw/day for 2 days (ASP group). The remaining rats were continued on their previous HSO or LSO diet (CON group). The amount of aspirin in the diet was precalculated for each rat from its daily food intake to provide 50 mg/Kg bw/day. Actual aspirin intake per Kg bw per day (mean  $\pm$  SEM, n=20) was 47.00 $\pm$ 2.74 mg in the HSO group and 48.17 $\pm$ 1.83 mg in the LSO group. Diets were provided fresh twice weekly, and tap water was provided fresh every day ad libitum. Animals were weighed weekly, and food intakes were recorded. Aspirin was purchased from Sigma (St. Louis, MO).

TABLE 2. Composition of diet

Ingredients	HSO	LSO
	g/100 g	
Casein <sup>a</sup>	16.8	16.8
l-Methionine <sup>a</sup>	0.2	0.2
Corn starch <sup>b</sup>	32.6	32.6
Sucrose <sup>c</sup>	32.6	32.6
Cellulose <sup>d</sup>	2.2	2.2
Salt mix <sup>e</sup>	4.4	4.4
Vitamin mix <sup>f</sup>	1.0	1.0
Safflower oil <sup>g</sup>	9.3	1.1
Beef tallow <sup>h</sup>	0.0	8.2
	mg/100 g	
PABA <sup>i</sup>	83.8	83.8
Inositol <sup>i</sup>	165.0	165.0
Cholinechloride <sup>j</sup>	331.8	331.8
$\alpha$ -Tocopherol <sup>j</sup>	212.8	212.8

<sup>a</sup>United States Biochemical Co., Cleveland, OH.

<sup>b</sup>Argo, Best Foods CPC International Inc., Englewood Cliffs, NJ.

<sup>c</sup>Californian and Hawaiian Sugar Co., San Francisco, CA.

<sup>d</sup>Teklad, Madison, WI.

<sup>e</sup>ICN Nutritional Biochemicals, Cleveland, OH, AIN  
Mineral Mixture 76; calcium phosphate dibasic 500.0 gm; sodium chloride 74.0 gm; potassium citrate monohydrate 220.0 gm; potassium sulfate 52.0 gm; magnesium oxide 24.0 gm; manganous carbonate 3.5 gm; ferric citrate 6.0 gm; zinc carbonate 1.6 gm; cupric carbonate 0.3 gm; potassium iodate 0.01 gm; sodium selenite 0.01 gm; chromium potassium sulfate 0.55 gm; sucrose, finely powdered 118.0 gm per Kg mixture.

<sup>f</sup>ICN Nutritional Biochemicals, Cleveland, OH., AIN  
Vitamin Mixture 76; thiamine HCl 600 mg; riboflavin 600 mg; pyridoxine HCl 700 mg; nicotinic acid 3 gm; d-calcium pantothenate 1.6 gm; d-biotin 20 gm; cyanocobalamin 1 mg; retinyl palmitate pre-mix (250,000 U/gm) 800 mg; dl- $\alpha$ -tocopheryl acetate pre-mix (25 U/gm) 20 gm; cholecalciferol (400,000 U/gm) 2.5 mg; menaquinone 5.0 mg; folic acid 200 mg; sucrose finely powdered 972.9 gm per Kg mixture.

<sup>g</sup>Hollywood Health Foods, Los Angeles, CA.

<sup>h</sup>Meat Lab., Dept. of Animal Sciences, Iowa State University.

<sup>i</sup>Nutritional Biochemicals, Cleveland, OH.

<sup>j</sup>Sigma, St. Louis, MO.

## Cell Isolation

During ether anesthesia, epididymal fat pads from 5 rats in the same diet group were removed and pooled. The mean (n=8), pooled epididymal fat pad weights were 10.4 g and 11.2 g in the HSO and LSO groups, respectively. The difference was not statistically significant. Part of the fat pad was stored to be used for fatty acid analysis; part of the fat pad was subjected to isolation of individual adipocytes using collagenase following the method of Rodbell (74). The fat pads were rinsed in Krebs Ringer Bicarbonate (KRB) buffer containing 3% bovine serum albumin (BSA), pH 7.4. The tissue pieces were then gently taken apart with forceps into small pieces, placed into 25 mL polypropylene beakers containing the following collagenase digestion mixture, 30 mg BSA and 1 mg collagenase per mL KRB. Two mL of the digestion mixture was used per g of tissue. The beakers were placed in a metabolic shaker at 37°C and gassed with 95% CO<sub>2</sub>-5% O<sub>2</sub>. The digestion mixture was filtered through a 250 μm nylon screen with KRB rinses. The crude cell suspension was centrifuged (50 x g for 1 min), washed 2 times and resuspended in KRB containing 3% BSA, pH 7.4. The number of isolated cells and their viability was determined microscopically using a hemacytometer and 0.01% trypan blue solution. In all cases, the cell viability was found to be greater than 95%. The volume of the cell suspension was adjusted to 25.0 mL which contained about  $1.3 \times 10^5$  to  $6.9 \times 10^5$  cells per mL suspension.

The microscopic cell counting method was verified for its accuracy by comparing its results with results of Coulter counter measurements of the same adipocyte preparation. For this comparison, one-month-old rats were fed either the HSO or LSO diet for 1 month; individual adipocytes were isolated, and counted by microscope using trypan blue dye and by Coulter counter after fixation of cells with  $\text{OsO}_4$  as follows: an aliquot of the cell suspension was fixed in 2%  $\text{OsO}_4$  in isotonic, 50 mM-collidine buffer, pH 7.4, for 48-72 h at room temperature (36). The fixed adipocytes were filtered through a 250  $\mu$  nylon screen, and the filtrate was refiltered through a 25  $\mu$  screen. Adipocytes on the top of a 25  $\mu$  screen were then taken up in a known volume of 0.9% NaCl-0.004% Triton X-100 for counting in a Coulter counter particle size analyzer using a 400  $\mu$  aperture. A model Z Coulter Counter was used. The two counting methods gave similar results, and statistical treatment showed a very close relationship between counts by the two methods ( $r=0.99$ ,  $P\leq 0.001$ ). The mean cell size ( $\mu$ , diameter) was 74.63 in the HSO group and 75.90 in the LSO group. The difference was not statistically significant. The cell size approximated the upper limit of accurate analysis using the microscopic technique. Greater cell size would have resulted in distortion of the cells in the counting chamber and inaccurate results.

Osmium tetroxide ( $\text{OsO}_4$ ) was obtained from Electron Microscopy Sciences (Fort Washington, PA). Nylon filtration screens were obtained from Tetko (Elmford, NY). Bovine serum albumin (Fraction V) was



obtained from Sigma (St. Louis, MO). Gas (95%O<sub>2</sub>-5% CO<sub>2</sub>) was obtained from Cooks Inc. (Algona, IA). Polypropylene flasks and other chemicals were obtained from Fisher Scientific Co. (Springfield, NJ).

## 2-Deoxy Glucose Transport

### Incubation time

The optimum incubation time for 2-DG transport into isolated adipocytes was decided in a preliminary experiment. As shown in Figure 2, the rate of 2-DG transport into adipocytes was increased linearly with time of incubation for a period of 15 min. After 15 min of incubation, the rate of 2-DG transport into adipocytes slowed, and the reaction curve became curvilinear suggesting that some efflux of 2-DG was occurring. Based on this result, cells were incubated for 10 min in the 2-deoxy glucose transport experiment.

### Incubation cell number

The cell number in the incubation mixture for optimum conditions for 2-DG transport study was also tested (Figure 3). Varied numbers of cells per mL of incubation mixture were incubated for 15 min, and 2-DG transport was measured. The results showed that 2-DG transport was increased linearly with the increase of the number of fat cells up to  $4.2 \times 10^5$  cells per mL of incubation mixture. Based on this result,  $4 \times 10^4$  to  $4 \times 10^5$  cells per mL of incubation mixture was desired in later

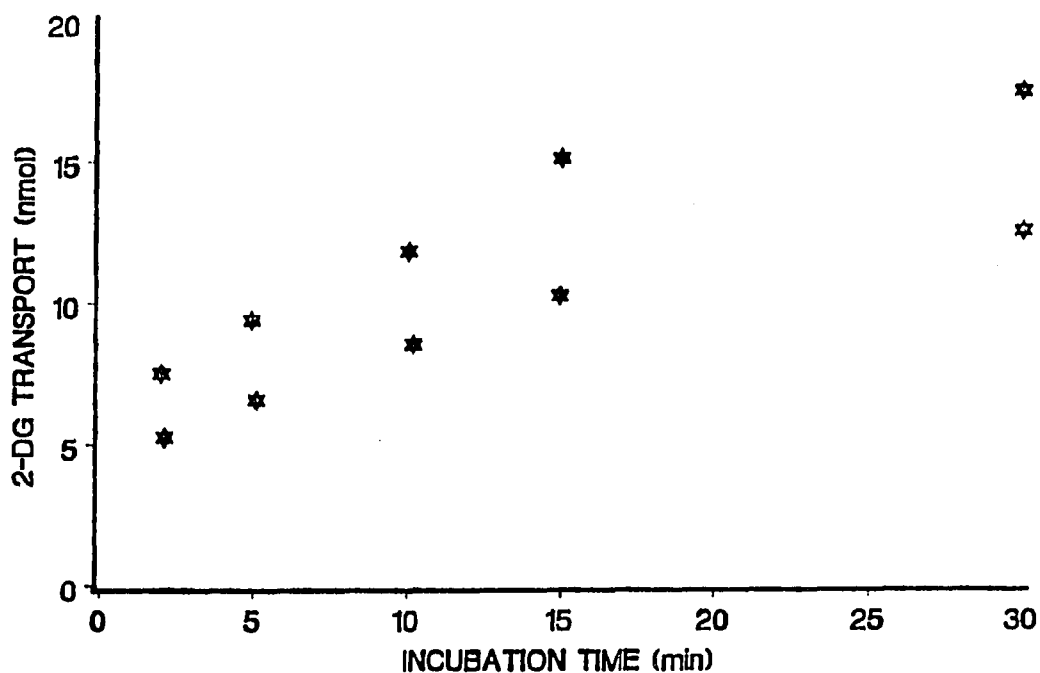


FIGURE 2. Effect of incubation time on adipocyte 2-deoxy glucose transport of rats.  $6.2 \times 10^5$  cells per ml were preincubated for 30 min and incubated at 37 C

experiments. Actually, in experiments, the incubation mixture contained  $3.9 \times 10^4$  to  $3 \times 10^5$  cells per ml.

#### 2-Deoxy glucose transport protocol

Six incubation flasks which contained 400  $\mu$ l of prepared cell suspension, 400  $\mu$ l KRB, 3% BSA, pH 7.4, with or without aspirin were placed in a metabolic shaker at 37°C for 30 min and gassed with 95% O<sub>2</sub>-5% CO<sub>2</sub>. Measurements of 2-DG transport were initiated by addition of test mixtures. By adding test mixtures, the final incubation

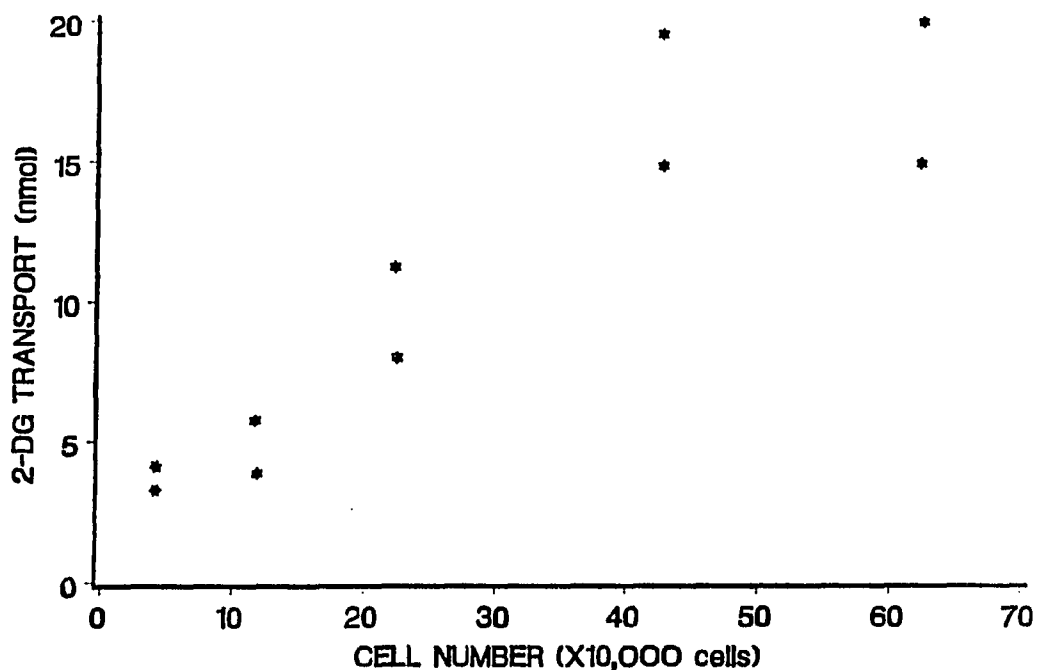


FIGURE 3. Effect of cell numbers on adipocyte 2-deoxy glucose transport of rats. Adipocytes were preincubated for 30 min and incubated for 15 min at 37°C

mixture contained 2-deoxy-D-(1-<sup>3</sup>H)-glucose at 2.0  $\mu$ Ci, inulin-(<sup>14</sup>C)carboxylic acid at 0.5  $\mu$ Ci, 2-deoxy-D-glucose (2-DG) at 0.1 mM and BSA at 30 mg/mL. The final incubation mixture also contained modifiers of eicosanoid synthesis. Norepinephrine at  $10^{-5}$  M, Asp at 23 mM, and antiserum to PGE at 167  $\mu$ l,<sup>5</sup> and combinations of these were used as modifiers of eicosanoid synthesis. The incubation flask without any modifiers of eicosanoids was used as control.

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<sup>5</sup>The amount of antiserum to PGE used was to bind most PGE produced in the cell incubation system as estimated from a preliminary experiment.

A sample of 200  $\mu$ l of incubation mixture was taken at 2, 5, and 10 min and layered on 1 mL silicone oil containing tubes. The reaction was stopped by separating adipocytes from the media according to the methods of Gliemann et al. (30). This was accomplished by centrifuging the tube at 3,000 x g for 1 min. Adipocytes were removed from atop the oil with a plastic-tipped automatic pipet and placed into scintillation vials. Fifteen mL of scintillation solution was added to each vial and radioactivity was determined by counting on a liquid scintillation counter (Tri-carb liquid scintillation Spectrometer, C2425, Packard Instrument Co., Downer's Grove, IL.). Extracellular space was calculated using  $^{14}\text{C}$ -inulin as the marker and used in subtracting 2-DG that was not transported into cells but trapped extracellularly. The mean, trapped 2-DG in extracellular space was  $15.93 \pm 8.91\%$  (mean  $\pm$  SEM) of the total amount of the radiolabelled 2-DG found in the packed cell at the 10 min incubation time. Reaction rates were plotted as nanomoles 2-DG transport per  $10^6$  cells vs. incubation time. The quenching effect of the sample in the liquid scintillation counting was corrected by using quenching curves developed with the fat cell incubation mixture.

Silicone oil ( $d=0.943$ ) was obtained from Aldrich (Milwaukee, WI). 2-deoxy-D-(1- $^3\text{H}$ )-glucose and inulin-( $^{14}\text{C}$ )-carboxylic acid were obtained from Amersham (Arlington Heights, IL). Two-deoxy-D-glucose, collagenase, and norepinephrine bitartrate were obtained from Sigma (St. Louis, MO). Scintivers Bio-HP and other chemicals were obtained from Fisher Scientific Co. (Springfield, NJ).

## Eicosanoid Analysis

Eicosanoids were quantified in adipocyte incubation mixtures. PGE<sub>1</sub>, PGE<sub>2</sub>, PGF<sub>2α</sub>, 6-keto-PGF<sub>1α</sub> (a stable product of PGI<sub>2</sub>), and TXB<sub>2</sub> (a stable product of TXA<sub>2</sub>) were measured by radioimmunoassay as described by McCosh et al. (58). Briefly, the method involved an overnight pre-precipitation of anti-rabbit gamma globulin with the specific antibody followed by a 24-h precipitation with sample and tritiated standard eicosanoids. Radioactivity in the precipitation was measured using a liquid scintillation counter and converted into amount of eicosanoids presented in the sample.

The PGE<sub>1</sub> antiserum had a cross reactivity of 10% with PGE<sub>2</sub>, and PGE<sub>2</sub> antiserum had a cross reactivity of 20% with PGE<sub>1</sub>. The other eicosanoid antisera did not show cross reactivities with each other. Parallelism of each eicosanoid in fat cell incubation mixtures was checked.

Tritiated PGE<sub>1</sub>, PGE<sub>2</sub>, PGF<sub>2α</sub>, and TXB<sub>2</sub> were purchased from New England Nuclear Co. (Boston, MA). Tritiated 6-keto-PGF<sub>1α</sub> was purchased from Amersham (Arlington Heights, IL). Standards were a gift from the Upjohn Company (Kalamazoo, MI).

## Fatty Acid Analysis

Extraction

Composition of total fatty acids were determined in the epididymal fat pads. Fatty acid extraction of tissue, prior to fatty acid methyl ester preparation, involved saponification of 50 mg epididymal adipose tissue with 2 mL of 10% KOH/EtOH at 60°C for 1 h. After the saponification, 2 mL of H<sub>2</sub>O was added, and saponifiable fatty acids were extracted three times with 4 mL portions of petroleum ether. The aqueous phase was then acidified with concentrated HCl, and fatty acids were extracted three times with 4 mL portions of petroleum ether. The combined fatty acid extracts were evaporated to dryness under nitrogen and methylated for 2 min with boron tri-fluoride methanol (BF<sub>3</sub>/MeOH at 14% W/V) at 100°C. The methylated fatty acids were extracted after addition of 1.0 mL distilled water and 2.0 mL hexane, and the organic phase was dried under nitrogen, and hexane (3 mL) was added to dissolve the methylated fatty acids.

Free fatty acids (FFA) were quantified in adipocyte incubation mixtures. The method consisted of a total lipid extraction using the Dole reagent (23) and a two-phase alcoholic extraction of fatty acids following extraction of non-polar and basic lipids. The total lipid extraction required 2 mL adipocyte incubation mixture, 60  $\mu$ l heptadecanoic acid solution (4 mg/mL, internal standard), 10 mL Dole reagent (heptane:isopropanol:1 N H<sub>2</sub>SO<sub>4</sub> = 1:4:0.1 by volume). After 5 min of vigorous shaking and 5 min of standing, 6.0 mL of heptane and

4.0 mL of water were added. The organic phase was separated by centrifugation at 3,000 x g, and the alcoholic phase was made basic with 1 N NaOH. The non-polar and basic lipids were extracted twice in heptane. The alcoholic phase was then acidified with 1 N HCl, and the FFA was extracted twice in heptane. The combined FFA extracts were subjected to fatty acid methyl ester preparation as described for fat pads. The final resuspended sample volume was 100  $\mu$ l for free fatty acid methyl esters. Heptadecanoic acid was obtained from Sigma (St. Louis, MO).  $\text{BF}_3/\text{MeOH}$  was obtained from Alltech (Deerfield, IL). Teflon linings were obtained from Arthur H. Thomas Co. (Philadelphia, PA). 2-Propanol, petroleum ether, and other reagents were obtained from Fisher (Springfield, NJ).

#### Gas chromatography

A Beckman Gas Chromatograph GC 72-5 equipped with a CRS-208 Columbia Scientific Integrator and a model 20 Texas Instruments Recorder was used for analysis of the fatty acid methyl esters. A 183 x 0.32 cm stainless steel column packed with Alltech 10% CS-10 on 100-120 WAW (Alltech, Deerfield, IL) was used. Instrument settings were as follows; column temperature, 180°C; injection port temperature, 220°C; detector temperature, 250°C; line temperature 220°C.

## Statistical Analysis

Data were analyzed statistically using Analysis of variance (ANOVA) and General Linear Models Procedure (GLM), and means were compared by Least Significant Difference (LSD) at a 5% level of probability. Means are reported with the standard error of the mean (SEM). Group means are reported with pooled SEM if the variance between treatment was homogeneous by Bartlett's test (85). There were large variabilities in glucose transport data from experiments of in vitro treatments. This might be caused by daily variability between adipocyte preparations. To eliminate this daily variability between adipocyte preparations, data of in vitro treatments of eicosanoid modifiers on 2-DG transport were expressed as the ratio to the control level within each preparation. Correlation coefficients were also determined and were deemed significant if the  $r \geq 0.5$  and  $p \leq 0.05$ .

## Preliminary Experiments

### Effect of norepinephrine on free fatty acid release

Adipocytes, isolated from epididymal fat pads of Zucker rats,<sup>6</sup> were incubated<sup>7</sup> with or without norepinephrine at a final concentration of  $10^{-5}$  M for 30 min. After a 30-min incubation, norepinephrine stimulated release of the total free fatty acids by 1.7-times (n=1) the

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<sup>6</sup>Rats were fed with Teklad laboratory rat chow containing 6% fat.

<sup>7</sup>See "Methods and Materials" for detail.



30-min control values and by 3.2-times the 0-min control values (Table 3).

Norepinephrine tended to increase absolute amounts of all individual free fatty acids, measured in the incubation mixture, except myristic and arachidonic acids during the 30-min incubation. Linoleic acid was increased by 1.5-times over 30-min control and by 2.2-times over 0-min control values. These results show that prostaglandin precursors are actively released during norepinephrine-treated lipolysis in vitro.

#### Effect of norepinephrine on eicosanoid synthesis

Amounts of eicosanoids, produced from isolated adipocytes during a 30 min incubation of isolated adipocytes with or without norepinephrine at  $10^{-5}$  M, were measured (n=1, values were estimated from range tests)<sup>8</sup> (Table 4).

At the basal level, isolated adipocytes produced PGE<sub>1</sub>, PGE<sub>2</sub>, TXB<sub>2</sub>, PGF<sub>2α</sub>, and PGI<sub>2</sub> (measured as 6 Keto-PGF<sub>1α</sub>) of a total of 215.1, 237.4, 93.6, 147.4, and 236.0 pg per 10<sup>6</sup> cells during a 30-min incubation. Norepinephrine-treated adipocytes produced PGE<sub>1</sub>, PGE<sub>2</sub>, PGF<sub>2α</sub> by 1.64-, 1.28-, and 1.4-times above the control levels. The synthesis of PGI<sub>2</sub> and TXB<sub>2</sub> were not changed by the norepinephrine treatment. These results suggest that norepinephrine stimulated the synthesis of eicosanoids by enhancing the availability of eicosanoid precursors via active lipolysis.

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<sup>8</sup>See "Methods and Materials" for detail.

TABLE 3. Free fatty acid (FFA) release of control (Con) and norepinephrine (Norepi) stimulated adipocytes<sup>a</sup>

FFA	Incubation time	30 min	
	0 min	Con	Norepi
		$\mu\text{g}/10^6$ cells	
14:0 <sup>b</sup>	4.1	30.2	8.9
16:0	208.8	711.3	1099.3
18:0	27.3	40.5	84.6
18:1	207.9	249.8	562.6
18:2	234.6	336.6	510.6
18:3	15.3	9.4	24.4
20:4	16.9	10.4	8.4
Total FFA	714.9	1388.2	2298.8

<sup>a</sup>n=1. Isolated adipocytes from epididymal fat pads were preincubated with or without norepinephrine ( $10^{-5}$  M) at 37degrees.C for 30 min.

<sup>b</sup>Number of carbons:number of double bonds.

TABLE 4. Eicosanoid synthesis in isolated rat adipocytes incubated with (Norepi) or without (Con) norepinephrine<sup>a</sup>

PG	PGE <sub>1</sub>	PGE <sub>2</sub>	TXB <sub>2</sub>	PGF <sub>2α</sub>	6KPGF <sub>1α</sub>
Treatment					
pg per 10 <sup>6</sup> cells					
Con x 0 min	158.4	194.7	70.8	122.6	178.2
Con x30 min	215.1	237.4	93.6	147.4	236.0
Norepi x30 min	353.3	303.8	99.8	203.3	237.2

<sup>a</sup>n=1. Values were estimated from range tests. Adipocytes were preincubated for 30 min and incubated with or without norepinephrine (10<sup>-5</sup> M) at 37°C.

#### Effect of aspirin dosage on eicosanoid synthesis

Six, two-months-old, male, Zucker rats were fed either high-fat (Hfat) or low-fat (Lfat) diet with aspirin for two days. Hfat diet contained 40% of calories from safflower oil as the only fat source, and the Lfat diet contained 2% of calories from safflower oil as the only fat source. Diets were formulated following the method of Dupont (25) to equalize nutrient density and were mixed with aspirin to provide either 50 mg/Kg/day or 100 mg/Kg/day. After 2 days' feeding, fat pads from 2 rats per group were pooled, fat cells were isolated,

preincubated for 30 min, and incubated for another 30 min. At the 30 min incubation time, PGE<sub>1</sub>, TXB<sub>2</sub>, and PGI<sub>2</sub> were measured in the incubation mixtures (n=1).<sup>9</sup> At the 30-min incubation time, isolated, control adipocytes produced 154.6 pg PGE<sub>1</sub>, 97.8 pg TXB<sub>2</sub>, and 172.5 pg PGI<sub>2</sub> per 10<sup>6</sup> cells.

Aspirin feeding caused inhibition of PGE<sub>1</sub> production by 34-64% and TXB<sub>2</sub> production by 53-72%. However, PGI<sub>2</sub> concentration in the incubation mixture was not changed by aspirin feeding. The higher dose of aspirin (100 mg/Kg/day) was not more effective than the lower dose (50 mg/Kg/day) in decreasing productions of eicosanoids. Based on these results, rats were fed with aspirin at 50 mg/Kg/day in later experiments. This dose of aspirin was reported as safe and does not interfere with the normal growth of weanling rats for a 15 days' feeding (60).

#### Preliminary feeding study

Eight, weanling Zucker rats were fed either Hfat or Lfat diets for 2 months. At the end of feeding, 2 rats/diet were fed aspirin at 50 mg/Kg/day for 2 days. Fat pads from two rats were pooled, and fat cells were isolated. Isolated fat cells were incubated with or without norepinephrine (10<sup>-5</sup> M) and anti-PGE (167 μl per 1,600 μl) plus norepinephrine (10<sup>-5</sup> M). PGE<sub>2</sub>, TXB<sub>2</sub>, and 2-DG transport were analyzed

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<sup>9</sup>See "Methods and Materials" for detail.

in cell incubation mixtures during a 30-min incubation.<sup>10</sup>

Effect of diet on adipocyte glucose transport Isolated adipocytes from the Lfat group had greater glucose transport than those from the Hfat group. At the 15-min incubation time, adipocytes from Lfat group transported 3.96 nanomoles (2.4-times the HSO, n=1) and adipocytes from Hfat group transported 1.63 nanomoles 2-DG into  $10^6$  cells. Supposedly, adipocytes from the Hfat group produced more eicosanoids than adipocytes from the Lfat group; the observed result was contradictory to several reports about stimulatory effects of prostaglandins on adipocyte glucose transport in vitro. Not only fat difference but also carbohydrate difference in the diet might affect the glucose transport into adipocytes. The Lfat diet contained approximately 1.5-times more carbohydrate by weight than the Hfat diet in compensating the low energy level of the Lfat diet. Lavau et al. (51) also reported similar, lower glucose transport rates of isolated adipocytes from rats fed a high-fat diet compared with the adipocytes from rats fed a low-fat diet. In their study, two diets provided almost the same amount of protein, and the energy level was equilibrated with contents of carbohydrates; the low-fat diet contained 70% carbohydrate by weight while the high-fat diet contained 10% carbohydrate by weight.

Based on this result, iso-nutrient diets varying only in fat qualities were used in later experiments. Two dietary fat qualities were to be used to alter eicosanoid precursor availabilities in

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<sup>10</sup>See "Methods and Materials" for detail.

adipocytes, thereby, to alter cellular production of eicosanoids.

Effect of aspirin feeding on adipocyte glucose transport

Aspirin feeding decreased glucose transport into isolated adipocytes in both diets compared with the control values. At the 15-min incubation time, this decrement was approximately 16% in the Hfat group and 38% in the Lfat group (n=1). The results suggest that the effect of endogenous eicosanoids on adipocyte glucose transport is in a positive direction.

Effects of norepinephrine and anti-PGE on adipocyte glucose transport

Norepinephrine, at a concentration of  $10^{-5}$  M, affected glucose transport into adipocytes very little compared to controls. At the 15-min incubation time, norepinephrine decreased glucose transport by only 2% in adipocytes from the Lfat group and 9% in adipocytes from the Hfat group (n=1).

On the other hand, anti-PGE clearly decreased glucose transport into adipocytes. At the 15-min incubation time, anti-PGE plus norepinephrine decreased glucose transport by 17% in Hfat group and by 27% in Lfat group. These results suggest that endogenous PGE enhanced glucose transport into adipocytes.

Effect of killing methods on eicosanoid synthesis

$PGE_2$  and  $TXB_2$  synthesis were measured in isolated adipocytes harvested from rats grown on either Hfat or Lfat diet during a 30-min incubation. As shown in Figure 4, the synthesis of  $PGE_2$  and  $TXB_2$  in

adipocytes were irregular to incubation time. During a 30-min incubation, PGE<sub>2</sub> synthesis was increased in the control (CON) groups and decreased in the norepinephrine (N) groups; TXB<sub>2</sub> synthesis was increased in the Hfat groups but decreased in the Lfat groups. The effect of norepinephrine on eicosanoid synthesis was also varied irregularly. At the 5-min incubation time, norepinephrine-treated adipocytes produced more PGE<sub>2</sub> compared with the controls in both diet groups (Hfat and Lfat) as expected. However at the 30-min incubation time, norepinephrine-treated adipocytes produced less PGE<sub>2</sub>, compared with the controls, in both diet groups. These results were different from previous results (Table 4); synthesis of PGE<sub>2</sub> and TXB<sub>2</sub> were consistently increased during a 30-min incubation, which was true for all other eicosanoids measured (PGE<sub>1</sub>, PGF<sub>2a</sub>, and 6-keto-PGF<sub>1a</sub>); norepinephrine-treated adipocytes produced more PGE<sub>2</sub> compared with the control during a 30-min incubation, which was also true for PGE<sub>1</sub> and PGF<sub>2a</sub> synthesis. The difference between the two experimental procedures was only the method of rat killing. In other times, epididymal fat pads were removed during light ether anesthesia. This time, epididymal fat pads were removed after decapitation. Based on this result, ether anesthesia rather than decapitation and exsanguination was used in later studies.

The difference between the two experimental procedures was only the method of rat killing. In other times, epididymal fat pads were removed during light ether anesthesia. This time, epididymal fat pads

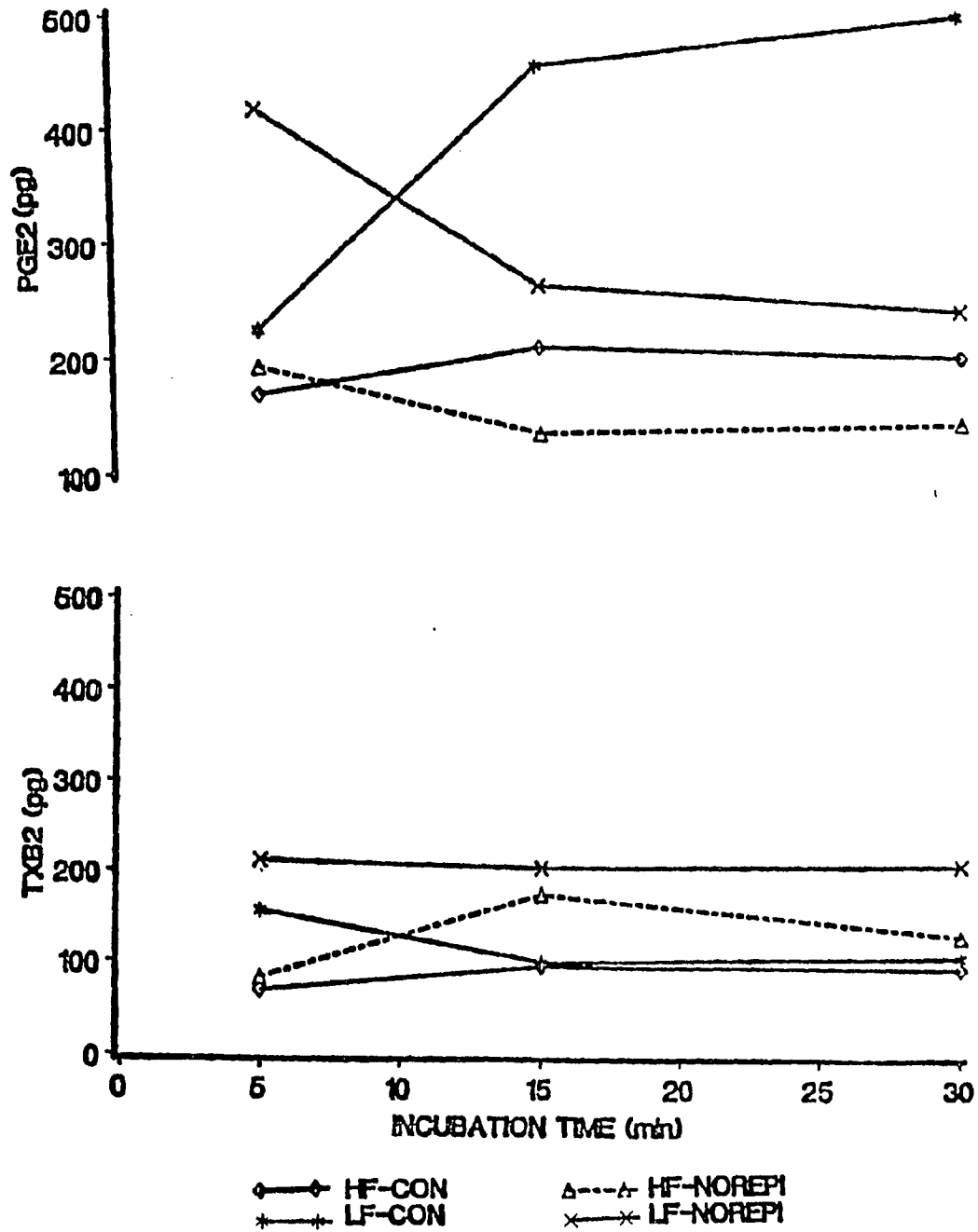


FIGURE 4. Eicosanoid synthesis in rat adipocytes -Effect of decapitation. pg eicosanoids/ $10^6$  cells. n=1. Isolated adipocytes from the epididymal fat pads were preincubated for 30 min and incubated at  $37^\circ$  with (Norepi) or without (Con) norepinephrine at  $10^{-5}$  M



were removed after decapitation. Based on this result, ether anesthesia rather than decapitation and exsanguination was used in later studies.

In this preliminary experiment, aspirin feeding decreased 2-DG transport into adipocytes and decreased PGE<sub>1</sub>, PGE<sub>2</sub>, TXB<sub>2</sub>, and PGF<sub>2α</sub>. Anti-PGE treatment in vitro also decreased glucose transport into adipocytes.

On the other hand, norepinephrine treatment in vitro did not show a clear effect on glucose transport into adipocytes. If free fatty acids were inhibiting glucose transport into cells as described in "Review of Literature", the observed results might come from certain substances which were produced during norepinephrine-treated lipolysis and which compensated for effects of free fatty acids in decreasing glucose transport.

These results suggest that endogenous eicosanoids may have a stimulatory role in glucose transport into adipocytes. Based on these results, a set of experiments was planned and performed to test the effects of endogenous eicosanoids on glucose transport into adipocytes. Herein the findings are reported.

## RESULTS

## Accumulated Food Intakes

The accumulated food intakes of rats fed the HSO or LSO diets for one month are presented in Figure 5.

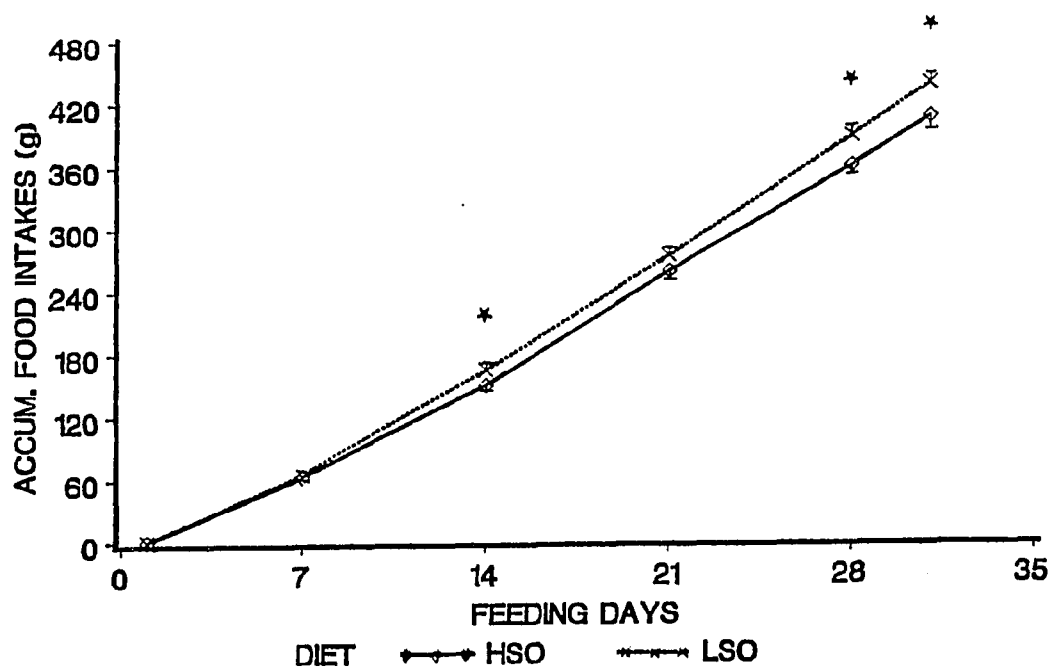


FIGURE 5. Accumulated food intakes of rats fed diets different in fat qualities. HSO diet contained 9.3% safflower oil and LSO diet contained 1.1% safflower oil and 8.2% beef tallow by weight. Mean  $\pm$  SEM. n=40. Values were significantly different between HSO and LSO: \*  $P < 0.05$

Rats fed the LSO diet ate more food than rats fed the HSO diet during the 31 feeding days. The mean daily food intakes of 40 rats per diet group were  $13.09 \pm 1.09$  g/day (Mean  $\pm$  SEM) for the HSO group and  $14.23 \pm 0.36$  g/day for the LSO group. The difference was statistically significant ( $P < 0.05$ ). As shown in Figure 5, the difference in accumulated food intakes between the two dietary groups was statistically significant at 14, 28, and 31 days of feeding. The mean, accumulated food intakes of rats in the HSO and LSO groups were  $146.12 \pm 4.94$  and  $165.88 \pm 5.64$  on the 14 day,  $359.30 \pm 7.06$  and  $381.18 \pm 8.81$  on the 28 day, and  $405.88 \pm 10.90$  and  $441.18 \pm 10.33$  on the 31 day of the feeding. The mean daily food intakes for the aspirin feeding period were  $15.53 \pm 3.90$  g/day for the HSO group and  $20.00 \pm 4.18$  g/day for the LSO group (Mean  $\pm$  SEM,  $n=40$ ). There was no significant difference in the accumulated food intakes or in the mean daily food intakes between control and aspirin feeding groups.

#### Body Weight Changes

The body weight changes of rats fed the HSO or the LSO diet for one month are presented in Figure 6. The mean growth rate of rats (Mean  $\pm$  SEM,  $n=40$ ) in the HSO group was  $5.52 \pm 0.11$  g/day and that of rats in the LSO group was  $5.57 \pm 0.18$  g/day during the 31 feeding days. The difference was not statistically significant. The mean growth rates (Mean  $\pm$  SEM,  $n=40$ ) for the aspirin feeding period were  $5.10 \pm 0.70$  g/day for the HSO group and  $3.97 \pm 0.63$  g/day for the LSO group. This difference was not statistically significant.

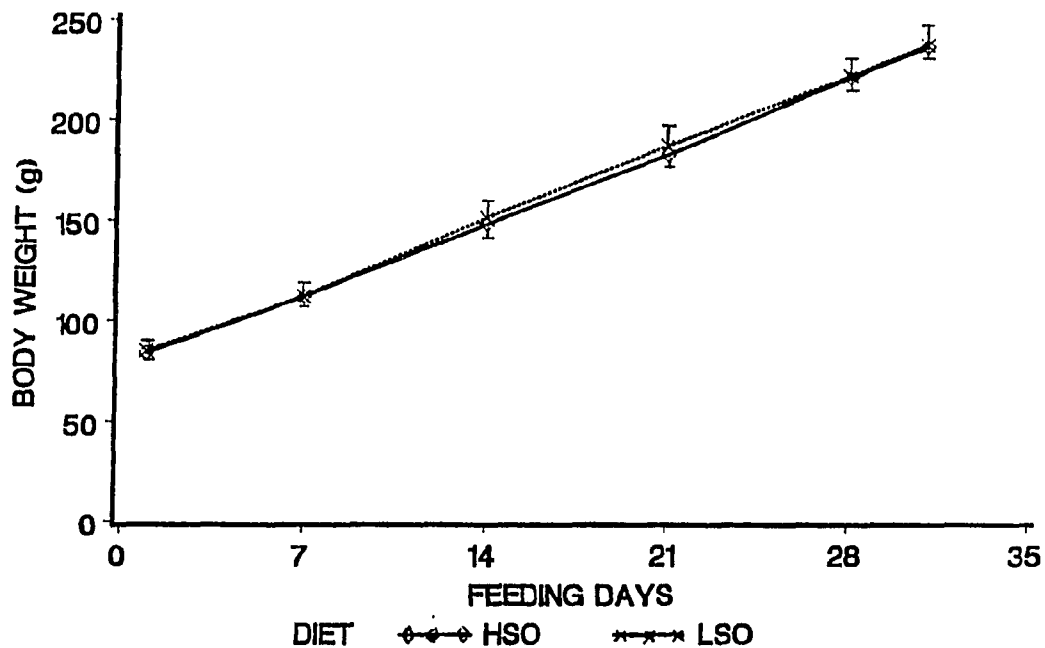


FIGURE 6. Body weight changes of rats fed diets different in fat qualities. HSO diet contained 9.3% safflower oil, LSO diet contained 1.1% safflower oil and 8.2% beef tallow by weight. Mean  $\pm$  SEM. n=40. None of the values were significantly different between HSO and LSO

#### Fatty Acid Composition of Epididymal Fat Pads

Fatty acid compositions as weight per cent of total fatty acids of epididymal fat pads are shown in Table 5. Dietary treatments markedly changed the composition of fatty acids in the epididymal fat pads. Fat

pads from rats fed the HSO diet contained significantly higher eicosanoid precursor fatty acids (linoleic and arachidonic acids) at the expense of other fatty acids (oleic, palmitic, stearic, myristic, linolenic acids). Epididymal fat pads from the HSO group contained 60.77% linoleic acid (7.5-times the LSO group), 18.14% oleic acid, 18.05% palmitic acid, and 0.71% arachidonic acid (14.2-times the LSO group). Epididymal fat pads from the LSO group contained 55.67% oleic acid (3.1-times the HSO group), 31.29% palmitic acid (1.7-times the HSO group), and 8.05% linoleic acid. All the observed differences in the composition of fatty acids between the two dietary groups were statistically significant. On the other hand, aspirin feeding (50 mg/Kg bw/day) for 2 days did not cause any significant difference in the fatty acid composition of epididymal fat pads compared with the control (data not shown).

#### Free Fatty Acid Release

Compositions of free fatty acids of norepinephrine-treated adipocytes during a 15-min incubation are shown in Table 6. Norepinephrine-treated adipocytes from rats fed HSO diet released significantly greater proportions of linoleic acid (61.96%, 5.8-times the LSO), less palmitic, oleic, and myristic acids (18.50%, 15.63%, and 0.85%; 0.68-, 0.28-, and 0.3-times the LSO group, respectively) during the 15-min incubation. Composition of free fatty acids released from norepinephrine-treated adipocytes during a 15-min incubation from rats fed the LSO diet was 55.75% oleic acid, 27.25% palmitic acid, and

TABLE 5. Effect of diets different in fat qualities on the fatty acid composition of epididymal fat pads of rats<sup>a</sup>

Fatty Acid	Diet	
	HSO	LSO
	weight per cent	
14:0 <sup>b</sup>	0.70 ± 0.05	2.15 ± 0.03*
16:0	18.05 ± 0.28	31.29 ± 0.81*
18:0	1.50 ± 0.06	2.32 ± 0.82*
18:1	18.14 ± 0.36	55.67 ± 0.59*
18:2	60.77 ± 0.49	8.05 ± 0.62*
18:3	0.16 ± 0.04	0.48 ± 0.04*
20:4	0.71 ± 0.03	0.05 ± 0.01*

<sup>a</sup>Mean ± SEM. n=8. HSO diet contained 9.3% safflower oil and LSO diet contained 1.1% safflower oil and 8.2% beef tallow by weight. Values were significantly different from HSO: \* P<0.05.

<sup>b</sup>Number of carbons:number of double bonds.

10.72% linoleic acid. Norepinephrine treatment did not cause any significant changes in the composition of free fatty acids compared to the control (data not shown).

There was no significant interaction in per cent of composition of each free fatty acid between diets (HSO, LSO) and groups (CON, ASP), diets and incubation conditions (0 min-Con, 15 min-Con, 15 min-Norepi), groups and incubation conditions, or among diets and groups and incubation conditions.

The effect of diets on the concentrations of free fatty acids released from isolated adipocytes are presented in Table 7. As shown in Table 7, adipocytes of HSO group released significantly greater amounts of stearic and linoleic acids than did adipocytes of the LSO group. Adipocytes of HSO group released  $44.25 \pm 5.27 \mu\text{g}$  stearic acid (1.7-times the LSO group), and  $1240.54 \pm 211.53 \mu\text{g}$  linoleic acid (12.1-times the LSO group) per  $10^6$  cells. Adipocytes of HSO group released greater amounts of arachidonic acid ( $11.61 \pm 4.91 \mu\text{g}/10^6$  cells, 2.8-times the LSO group), but the difference was not statistically significant. On the other hand, adipocytes of the LSO group released significantly greater amount of oleic acid ( $531.87 \pm 57.93 \mu\text{g}$ , 1.7-times the HSO group) per  $10^6$  cells. Adipocytes of HSO group released significantly ( $P < 0.05$ ) greater amounts of total free fatty acids than adipocytes of LSO group (HSO,  $2002.17 \pm 391.73 \mu\text{g}/10^6$  cells, 2.1-times the LSO group).

TABLE 6. Effect of diets different in fat qualities on the composition of free fatty acids of rat adipocytes<sup>a</sup>

FFA	Diet	
	HSO	LSO
	weight per cent	
14:0 <sup>b</sup>	0.85 ± 0.08	2.54 ± 0.21*
16:0	18.50 ± 1.00	27.25 ± 1.24*
18:0	2.21 ± 0.51	2.66 ± 0.51
18:1	15.63 ± 0.66	55.75 ± 0.63*
18:2	61.96 ± 1.21	10.72 ± 1.33*
18:3	0.27 ± 0.12	0.65 ± 0.15
20:4	0.58 ± 0.13	0.43 ± 0.31

<sup>a</sup>Mean ± SEM. n=8. HSO diet contained 9.3% safflower oil, and LSO diet contained 1.1% safflower oil and 8.2% beef tallow by weight. Adipocytes were isolated from epididymal fat pads of rats, preincubated for 30 min and incubated with norepinephrine (10<sup>-5</sup> M) for 15 min at 37°C. Values were significantly different from HSO: P<0.05.  
from HSO: \* P<0.05.

<sup>b</sup>Numbers of carbons:numbers of double bonds.



TABLE 7. Effect of diets different in fat qualities on the concentration of free fatty acids released from rat adipocytes<sup>a</sup>

Free Fatty Acid	Diet	
	HSO	LSO
	$\mu\text{g}/10^6$ cells	
14:0 <sup>b</sup>	17.02 $\pm$ 1.07	24.23 $\pm$ 4.74
16:0	370.40 $\pm$ 81.16	259.97 $\pm$ 48.33
18:0	44.25 $\pm$ 5.27	25.38 $\pm$ 4.97*
18:1	312.94 $\pm$ 124.41	531.87 $\pm$ 57.93*
18:2	1240.54 $\pm$ 211.53	102.27 $\pm$ 15.52*
18:3	5.41 $\pm$ 0.71	6.20 $\pm$ 1.45
20:4	11.61 $\pm$ 4.91	4.10 $\pm$ 0.32
Total	2002.17 $\pm$ 391.73	954.02 $\pm$ 124.28*

<sup>a</sup>Mean  $\pm$  SEM. n=8. HSO diet contained 9.3% safflower oil, and LSO diet contained 1.1% safflower oil and 8.2% beef tallow by weight. Adipocytes were isolated from epididymal fat pads of rats, preincubated for 30 min, and incubated with norepinephrine ( $10^{-5}$  M) for 15 min. Values were significantly different from HSO: \*  $P \leq 0.05$ .

<sup>b</sup>Number of carbons:number of double bonds.

The release of total free fatty acids in aspirin-fed group was  $983.5 \pm 149.9 \mu\text{g}$ , and that in the control group was  $1748.5 \pm 215.5 \mu\text{g}$  ( $P < 0.05$ ) per  $10^6$  cells ( $n=24$ ). On the other hand, aspirin feeding did not alter the composition of released free fatty acids compared with the control group.

The effect of incubation conditions (incubation time and norepinephrine treatment) on the concentrations of free fatty acids released from isolated adipocytes is presented in Table 8. Even though some fatty acids were released in greater average amounts during a 15-min incubation or by the norepinephrine treatment compared with the control, none of the differences were statistically significant. The concentration of total free fatty acids released from adipocytes of 0 min-control (0 min, Con), 15 min-Control (15 min, Con), and 15 min-norepinephrine (15 min, Norepi) was  $1346.1$ ,  $1273.7$ , and  $1478.1 \mu\text{g}/10^6$  cells. There was no interaction in the concentration of each free fatty acid between diets (HSO, LSO) and groups (CON, ASP), diets and incubation conditions (0 min-Con, 15 min-Con, 15 min-Norepi), groups and incubation conditions, or among diets and groups and incubation conditions.

#### 2-Deoxy Glucose Transport

Figure 7 and Figure 8 show effect of diets and aspirin feeding on 2-deoxy glucose (2-DG) transport into isolated adipocytes. At the end of one-month feeding, rats were fed diets with (CON) or without aspirin (ASP, 50 mg/Kg bw/day) for 2 days ad libitum. Isolated adipocytes were

TABLE 8. Effect of incubation conditions on the concentration of free fatty acids of rat adipocytes<sup>a</sup>

FFA	Incubation condition			SEM
	0 min		15 min	
	Con	Con	Norepi	
	$\mu\text{g}/10^6$ cells			
14:0 <sup>b</sup>	17.86	15.55	17.07	3.39
16:0	292.87	301.43	336.24	49.86
18:0	29.70	35.90	29.73	5.93
18:1	325.97	345.73	428.98	59.08
18:2	663.03	559.76	647.36	131.40
18:3	6.25	5.10	4.75	2.12
20:4	10.42	10.23	13.96	3.44
Total	1346.10	1273.70	1478.10	194.10

<sup>a</sup>n=16. Adipocytes were isolated from epididymal fat pads, preincubated for 30 min, and incubated with (Norepi) or without (Con) norepinephrine at  $10^{-5}$  M at 37°C. None of the values were significantly different in rows.

<sup>b</sup>Number of carbons:number of double bonds.

preincubated with KRB buffer containing 3% BSA for 30 min prior to addition of radiolabeled glucose.

As shown in Figure 7, adipocytes from rats fed the HSO diet had significantly greater 2-DG transport compared with adipocytes from the LSO-fed rats during a 10-min incubation. Nanomoles (Mean  $\pm$  SEM, n=48) of 2-DG transport into  $10^6$  adipocytes of HSO and LSO groups were  $4.48 \pm 0.43$  and  $2.96 \pm 0.19$  at 2-min ( $P < 0.05$ ),  $8.66 \pm 0.79$  and  $6.31 \pm 0.54$  at 5-min ( $P < 0.05$ ), and  $12.38 \pm 1.18$  and  $10.22 \pm 1.17$  at 10-min ( $P < 0.05$ ) incubation times, respectively.

Aspirin feeding (50 mg/Kg/day, 2 days) did not change 2-DG transport into adipocytes significantly compared with the control group.

Nanomoles (Mean  $\pm$  SEM, n=48) of 2-DG transport into  $10^6$  adipocytes of control rats and aspirin fed rats were  $3.34 \pm 0.30$  and  $4.11 \pm 0.38$  at 2-min,  $7.07 \pm 0.74$  and  $7.90 \pm 0.66$  at 5-min, and  $12.02 \pm 1.38$  and  $10.11 \pm 0.77$  at 10-min incubation times, respectively.

Effects of in vitro treatments within each diet group are presented in Tables 9-11. A large variability was found in the 2-DG transport data of in vitro treatments of control (Con), norepinephrine (N), anti-PGE (AntiE), anti-PGE plus norepinephrine (AntiEN), aspirin (Asp), and aspirin plus norepinephrine (AspN) between adipocyte preparations. When 2-DG transport data (nanomoles 2-DG transport into  $10^6$  cells during a 10 min incubation) were treated statistically to compare effects of in vitro treatments, no effects were significant.

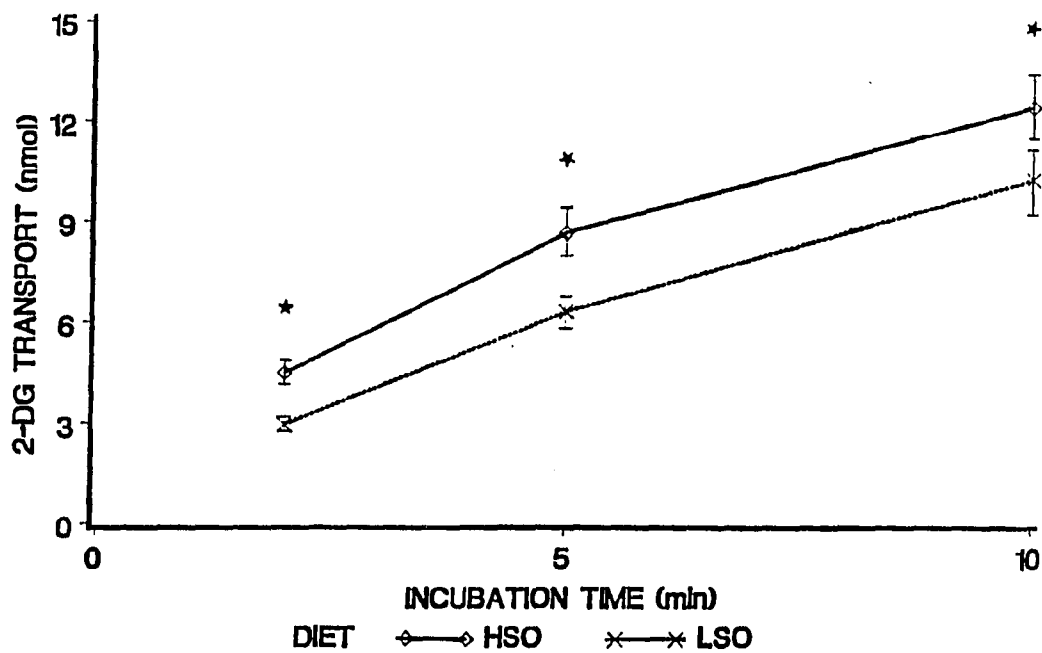


FIGURE 7. Effects of diets on adipocyte 2-deoxy glucose transport. Mean  $\pm$  SEM.  $n=48$ . Nanomoles 2-DG transport into  $10^6$  cells during a 10 min incubation. Rats were fed diets with 9.3% safflower oil (HSO) or 1.1% safflower oil plus 8.2% beef tallow for 1 mon. Adipocytes were isolated from epididymal fat pads, preincubated 30 min, and incubated for 10 min at  $37^\circ$ . Values were different between HSO and LSO: \*  $P < 0.05$

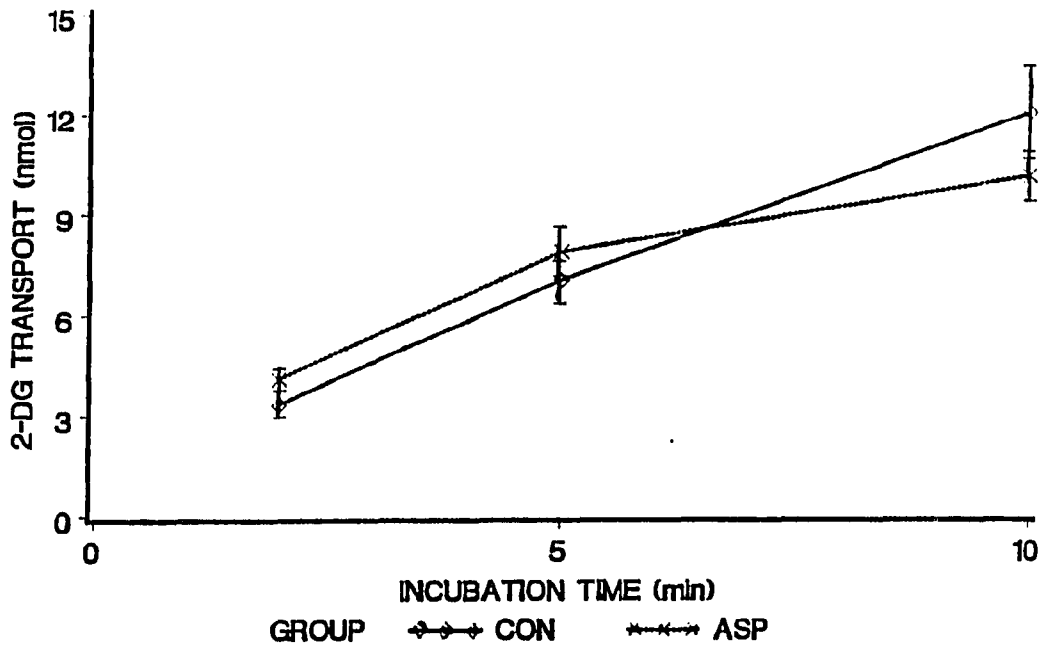


FIGURE 8. Effects of aspirin feeding on adipocyte 2-deoxy glucose transport. Mean  $\pm$  SEM.  $n=48$ . Nanomoles 2-DG transport into  $10^6$  cells during a 10 min incubation. Rats were fed diets with (ASP) or without (CON) aspirin at 50 mg/Kg bw/day for 2 days. Adipocytes were isolated from epididymal fat pads, preincubated 30 min, and incubated for 10 min at 37°C. None of the values were significantly different between CON and ASP

This statistical insignificance might be caused by the daily variabilities among adipocyte preparations. To best demonstrate the effect of in vitro treatments of those eicosanoid modifiers on 2-DG transport into adipocytes and to eliminate the daily variabilities among adipocyte preparations, 2-DG transport data were converted into ratio to the control (control=1.00) in each diet group and analyzed statistically.

The treatment of norepinephrine at  $10^{-5}$  M decreased 2-DG transport into adipocytes, compared with the control, in the HSO-CON group. In the HSO-CON group, at the 2-min incubation time, norepinephrine, norepinephrine plus aspirin (23 mM), or norepinephrine plus anti-PGE (167  $\mu$ l per 1,600  $\mu$ l final incubation mixture) decreased 2-DG transport compared with the control. At the 5-min incubation time, norepinephrine, norepinephrine plus aspirin, and norepinephrine plus anti-PGE tended to decrease 2-DG transport compared with the control, but not significantly. At the 10-min incubation time, norepinephrine tended to decrease 2-DG transport. When norepinephrine was treated along with aspirin or anti-PGE, the decrement in 2-DG transport became statistically significant. On the other hand none of the norepinephrine treatments significantly affected 2-DG transport into adipocytes in other diet groups in vitro.

Effects of in vitro aspirin treatments (Asp, 23 mM) in each diet group are shown in Table 10. Aspirin treatment at 23 mM decreased 2-DG transport into adipocytes compared with the control in the HSO-CON

TABLE 9. Effect of norepinephrine on adipocyte 2-deoxy glucose transport in vitro<sup>a</sup>

Treatment	N	AspN	AntiEN
Diet group			
ratio to control			
2 min			
HSO-CON <sup>b</sup>	0.56 ± 0.15 <sup>B</sup>	0.50 ± 0.11 <sup>B</sup>	0.65 ± 0.13 <sup>B</sup>
HSO-ASP	0.96 ± 0.05 <sup>A</sup>	1.05 ± 0.33 <sup>A</sup>	0.86 ± 0.08 <sup>A</sup>
LSO-CON	1.41 ± 0.34 <sup>A</sup>	1.12 ± 0.19 <sup>A</sup>	0.79 ± 0.23 <sup>A</sup>
LSO-ASP	0.94 ± 0.22 <sup>A</sup>	0.92 ± 0.15 <sup>A</sup>	0.74 ± 0.07 <sup>A</sup>
5 min			
HSO-CON	0.80 ± 0.12 <sup>A</sup>	0.93 ± 0.26 <sup>A</sup>	0.89 ± 0.32 <sup>B</sup>
HSO-ASP	0.89 ± 0.20 <sup>A</sup>	0.87 ± 0.13 <sup>A</sup>	0.84 ± 0.11 <sup>A</sup>
LSO-CON	1.04 ± 0.30 <sup>A</sup>	1.12 ± 0.20 <sup>A</sup>	0.71 ± 0.08 <sup>A</sup>
LSO-ASP	1.05 ± 0.06 <sup>A</sup>	1.19 ± 0.11 <sup>A</sup>	1.48 ± 0.49 <sup>A</sup>
10 min			
HSO-CON	0.90 ± 0.11 <sup>A</sup>	0.74 ± 0.09 <sup>B</sup>	0.74 ± 0.08 <sup>B</sup>
HSO-ASP	0.92 ± 0.16 <sup>A</sup>	1.02 ± 0.11 <sup>A</sup>	0.91 ± 0.11 <sup>A</sup>
LSO-CON	1.47 ± 0.50 <sup>A</sup>	1.43 ± 0.64 <sup>A</sup>	1.02 ± 0.16 <sup>A</sup>
LSO-ASP	1.70 ± 0.37 <sup>A</sup>	1.77 ± 0.52 <sup>A</sup>	1.60 ± 0.32 <sup>A</sup>

<sup>a</sup>n=4. Mean ± SEM. The nanomoles of 2-DG transport/10<sup>6</sup> cells of control treatments (ratio=1.00) in diet groups (HSO-CON, HSO-ASP, LSO-CON, and LSO-ASP) at 2-, 5-, and 10-min incubation times are: 2-min, 5.03 ± 1.38, 5.92 ± 2.22, 2.93 ± 0.54, and 3.41 ± 0.89; 5-min, 10.54 ± 3.87, 9.85 ± 2.79, 5.49 ± 1.06, and 6.31 ± 1.55; 10-min, 16.41 ± 6.43, 12.01 ± 3.57, 7.54 ± 1.47, and 6.45 ± 2.07. The letter B as a superscript shows a significant difference from the control: P<0.05.

<sup>b</sup>Rats were fed diets with 9.3% safflower oil (HSO) or 1.1% safflower oil plus 8.2% beef tallow (LSO) by weight. At the end of 1 mon feeding, rats were fed diets with (ASP) or without (CON) aspirin (50mg/Kg bw/day) for 2 days. Isolated adipocytes from epididymal fat pads were preincubated for 30 min and incubated with norepinephrine (N) (10<sup>-5</sup> M), or aspirin (23 mM) plus norepinephrine (10<sup>-5</sup> M) (AspN), or Anti-PGE (167 μL/1200 μL) plus norepinephrine (10<sup>-5</sup> M) (AntiEN).



group. In HSO-CON group, aspirin treatment tended to decrease 2-DG transport compared with the control consistently during a 10-min incubation. The decrement was statistically significant at the 10-min incubation time. In HSO-CON group, aspirin plus norepinephrine also tended to decrease 2-DG transport consistently during a 10-min incubation. The decrements in 2-DG transport by aspirin plus norepinephrine were significant at the 2- and 10-min incubation times. On the other hand, aspirin plus norepinephrine significantly increased 2-DG transport compared with the control in the LSO-ASP group at the 10-min incubation time. None of the aspirin treatments significantly affected 2-DG transport into adipocytes in the HSO-ASP group or in the LSO-CON group.

Effects of in vitro anti-PGE on 2-DG transport into isolated adipocytes in each diet group are shown in Table 11.

Anti-PGE (167  $\mu$ l per 1,600  $\mu$ l final incubation mixture) decreased 2-DG transport into adipocytes compared with the control in the HSO-CON group. The decrement was statistically significant at an early time point; 2-min incubation time. Anti-PGE plus norepinephrine significantly decreased 2-DG transport into adipocytes at 2- and 10-min incubation times. None of the anti-PGE treatments significantly affected 2-DG transport into isolated adipocytes in other diet groups.

TABLE 10. Effect of aspirin on adipocyte 2-deoxy glucose transport in vitro<sup>a</sup>

Treatment	Asp	AspN
Diet group		
ratio to control		
2 min		
HSO-CON <sup>b</sup>	0.98 ± 0.15 <sup>A</sup>	0.50 ± 0.11 <sup>B</sup>
HSO-ASP	1.07 ± 0.30 <sup>A</sup>	1.05 ± 0.33 <sup>A</sup>
LSO-CON	1.23 ± 0.25 <sup>A</sup>	1.12 ± 0.19 <sup>A</sup>
LSO-ASP	0.69 ± 0.11 <sup>A</sup>	0.92 ± 0.15 <sup>A</sup>
5 min		
HSO-CON	0.79 ± 0.17 <sup>A</sup>	0.93 ± 0.26 <sup>A</sup>
HSO-ASP	0.85 ± 0.05 <sup>A</sup>	0.87 ± 0.13 <sup>A</sup>
LSO-CON	1.34 ± 0.37 <sup>A</sup>	1.12 ± 0.20 <sup>A</sup>
LSO-ASP	1.05 ± 0.08 <sup>A</sup>	1.19 ± 0.11 <sup>A</sup>
10 min		
HSO-CON	0.78 ± 0.10 <sup>B</sup>	0.74 ± 0.08 <sup>B</sup>
HSO-ASP	1.11 ± 0.05 <sup>A</sup>	1.02 ± 0.11 <sup>A</sup>
LSO-CON	1.54 ± 0.26 <sup>A</sup>	1.43 ± 0.34 <sup>A</sup>
LSO-ASP	1.56 ± 0.27 <sup>A</sup>	1.77 ± 0.32 <sup>B</sup>

<sup>a</sup>n=4. Mean ± SEM. The nanomoles of 2-DG transport/10<sup>6</sup> cells control treatments (ratio=1.00) in each diet group (HSO-CON, HSO-ASP, LSO-CON, and LSO-ASP) at 2-, 5-, and 10-min incubation times are: 2-min, 5.03 ± 1.38, 5.92 ± 2.22, 2.93 ± 0.54, and 3.41 ± 0.89; 5-min, 10.54 ± 3.87, 9.85 ± 2.79, 5.49 ± 1.06, and 6.31 ± 1.55; 10-min, 16.41 ± 6.43, 12.01 ± 3.57, 7.54 ± 1.47, and 6.45 ± 2.07. The letter B as a superscript shows a significant difference from the control: P<0.05.

<sup>b</sup>Rats were fed diets with 9.3% safflower oil (HSO) or 1.1% safflower oil plus 8.2% beef tallow (LSO) by weight. At the end of 1 mon feeding, rats were fed diets with (ASP) or without (CON) aspirin at 50 mg/Kg bw/day for 2 days. Isolated adipocytes from epididymal fat pads were preincubated for 30 min and incubated with <sub>5</sub> aspirin (23 mM, Asp) or aspirin (23 mM) plus norepinephrine (10<sup>-5</sup> M).

TABLE 11. Effect of anti-PGE on adipocyte 2-deoxy glucose transport in vitro<sup>a</sup>

Treatment	AntiE	AntiEN
Diet group		
ratio to control		
2 min		
HSO-CON <sup>b</sup>	0.55 ± 0.16 <sup>B</sup>	0.65 ± 0.13 <sup>B</sup>
HSO-ASP	1.23 ± 0.32 <sup>A</sup>	0.86 ± 0.08 <sup>A</sup>
LSO-CON	0.96 ± 0.11 <sup>A</sup>	0.79 ± 0.23 <sup>A</sup>
LSO-ASP	0.91 ± 0.11 <sup>A</sup>	0.74 ± 0.12 <sup>A</sup>
5 min		
HSO-CON	0.94 ± 0.14 <sup>A</sup>	0.89 ± 0.23 <sup>A</sup>
HSO-ASP	0.89 ± 0.04 <sup>A</sup>	0.84 ± 0.11 <sup>A</sup>
LSO-CON	0.99 ± 0.22 <sup>A</sup>	0.71 ± 0.24 <sup>A</sup>
LSO-ASP	0.90 ± 0.15 <sup>A</sup>	1.48 ± 0.49 <sup>A</sup>
10 min		
HSO-CON	0.95 ± 0.10 <sup>A</sup>	0.74 ± 0.08 <sup>B</sup>
HSO-ASP	0.89 ± 0.08 <sup>A</sup>	0.91 ± 0.11 <sup>A</sup>
LSO-CON	1.63 ± 0.24 <sup>A</sup>	1.02 ± 0.16 <sup>A</sup>
LSO-ASP	1.67 ± 0.21 <sup>A</sup>	1.60 ± 0.32 <sup>A</sup>

<sup>a</sup>n=4. Mean ± SEM. The nanomoles of 2-DG transport/10<sup>6</sup> cells of control treatments (ratio=1.00) in diet groups (HSO-CON, HSO-ASP, LSO-CON, and LSO-ASP) at 2-, 5-, and 10-min incubation times are: 2-min, 5.03 ± 1.38, 5.92 ± 2.22, 2.93 ± 0.54, and 3.41 ± 0.89; 5-min, 10.54 ± 3.87, 9.85 ± 2.79, 5.49 ± 1.06, and 6.31 ± 1.55; 10-min, 16.41 ± 6.43, 12.01 ± 3.57, 7.54 ± 1.47, and 6.45 ± 2.07. The letter B as a superscript shows a significant difference from the control: P<0.05.

<sup>b</sup>Rats were fed diets with 9.3% safflower oil (HSO) or 1.1% safflower oil plus 8.2% beef tallow (LSO) by weight. At the end of 1 mon feeding, rats were fed diets with (ASP) or without (CON) aspirin (50 mg/Kg bw/day) for 2 days. Isolated adipocytes from epididymal fat pads were preincubated for 30 min and incubated without (control) or with Anti-PGE (167 μL per 1600 μL final incubation mixture) or Anti-PGE plus norepinephrine (10<sup>-5</sup> M) (AntiEN).

### Correlation Coefficients

Correlation coefficients were estimated between per cent compositions of fatty acids in epididymal fat pads and per cent compositions of free fatty acids released from isolated adipocytes. Correlation coefficients also were estimated between rates of 2-DG transport into adipocytes during a 5-min incubation (nanomoles 2-DG transport per min) and rates of free fatty acid release during a 15-min incubation ( $\mu\text{g}$  FFA per min) in adipocytes (Table 12).

The content of each fatty acid in the epididymal fat pads was positively correlated with the content of the same fatty acid released from isolated adipocytes at the 0-min incubation time (14:0,  $r=+0.76$ ,  $P=0.0001$ ; 16:0,  $r=+0.92$ ,  $P=0.0001$ ; 18:0,  $r=+0.56$ ,  $P=0.0001$ ; 18:1,  $r=+0.99$ ,  $P=0.0001$ ; 18:2,  $r=+0.99$ ,  $P=0.0001$ ; 18:3,  $r=+0.78$ ,  $P=0.0001$ ;  $n=96$ ).

The release of linoleic acid ( $\mu\text{g}$ ) from adipocytes was positively correlated with 2-DG transport in the HSO-CON group ( $r=+0.56$ ,  $P=0.0153$ ,  $n=24$ ) and in the LSO-ASP group ( $r=+0.74$ ,  $P=0.0004$ ,  $n=24$ ). The release of arachidonic acid was positively correlated with 2-DG transport in the HSO-CON group ( $r=+0.63$ ,  $P=0.0265$ ,  $n=24$ ). The release of linolenic acid was negatively correlated with 2-DG transport in the HSO-CON, HSO-ASP, and LSO-ASP groups (HSO-CON,  $r=-0.72$ ,  $P=0.0007$ ; HSO-ASP,  $r=-0.94$ ,  $P=0.0001$ ; LSO-ASP,  $r=-0.52$ ,  $P=0.0813$ ;  $n=24$ ). The release of oleic acid was positively correlated with 2-DG transport in the HSO-CON ( $r=+0.72$ ,  $P=0.0007$ ,  $n=24$ ) and LSO-ASP ( $r=+0.56$ ,  $P=0.0162$ ,  $n=24$ ) groups. The

TABLE 12. Correlation coefficients between free fatty acid release and 2-deoxy glucose transport<sup>a</sup>

FFA		14:0	16:0	18:0	18:1	18:2	18:3	20:4	Total FFA
Diet Group									
HSO-CON	r	+0.28	+0.40	-0.12	+0.72	+0.56	-0.72	+0.63	+0.60
	P	.0265	.1006	.6253	<u>.0007</u>	<u>.0153</u>	<u>.0007</u>	<u>.0265</u>	<u>.0090</u>
	n	24	24	24	24	24	24	24	24
HSO-ASP	r	+0.78	-0.52	+0.61	+0.47	+0.04	-0.94	+0.49	-0.04
	P	<u>.0001</u>	<u>.0261</u>	<u>.0072</u>	.0467	.8649	<u>.0001</u>	.0107	.8619
	n	24	24	24	24	24	24	24	24
LSO-CON	r	+0.11	+0.15	+0.28	+0.10	+0.13	+0.42	-0.41	-0.13
	P	.6559	.5540	.2629	.6885	.6123	.1726	.0920	.6148
	n	24	24	24	24	24	24	24	24
LSO-ASP	r	-0.31	+0.66	+0.60	+0.56	+0.74	-0.52	+0.05	+0.61
	P	.2082	<u>.0030</u>	<u>.0084</u>	<u>.01620</u>	<u>.0004</u>	.0813	.8390	<u>.0068</u>
	n	24	24	24	24	24	24	24	24

<sup>a</sup>Correlation coefficients were estimated between rates of 2-DG transport into adipocytes during a 5-min incubation (nanomoles 2-DG transport per min) and rates of free fatty acid release during a 15-min incubation ( $\mu\text{g}$  FFA per min) by adipocytes. Rats were fed diets with 9.3% safflower oil (HSO) or 1.1% safflower oil plus 8.2% beef tallow (LSO) by weight. At the end of 1 mon feeding, half of the rats were fed diets (either HSO or LSO) with (ASP) or without (CON) aspirin (50mg/Kg bw/day) for 2 days. Isolated adipocytes from epididymal fat pads were preincubated for 30 min and incubated for 15 min at 37°C.

release of stearic acid was positively correlated with 2-DG transport in the HSO-ASP group ( $r=+0.61$ ,  $P=0.0072$ ,  $n=24$ ). The release of palmitic acid was negatively correlated with 2-DG transport in the HSO-ASP group ( $r=-0.52$ ,  $P=0.0261$ ,  $n=24$ ), but positively correlated in the LSO-ASP group ( $r=+0.66$ ,  $P=0.0030$ ,  $n=24$ ). The release of myristic acid was positively correlated with 2-DG transport in the HSO-ASP group ( $r=+0.78$ ,  $P=0.0001$ ,  $n=24$ ). The release of total free fatty acids were positively correlated with 2-DG transport in the HSO-CON group and the LSO-ASP group (HSO-CON,  $r=+0.60$ ,  $P=0.0090$ ; LSO-ASP,  $r=+0.61$ ,  $P=0.0008$ ;  $n=24$ ).

## DISCUSSION

Because stored fatty acids in adipose tissue reflect the dietary fatty acid composition, investigators have used this tissue to study effects of different diets on eicosanoid production or intracellular metabolism affected by endogenous eicosanoids. Isolated adipocytes were used as a model to study the relationship between endogenous eicosanoid production and glucose transport into cells. The objective was to determine if the dietary fatty acids actually were reflected in the composition of fatty acids in the epididymal fat pads, if the composition of fatty acids in the fat pads were reflected in the composition of fatty acids released from isolated adipocytes; and consequently, if the amounts of free fatty acids in adipocytes would affect glucose transport by isolated adipocytes. Three eicosanoid modifiers were used; aspirin (feeding and treatment in vitro) to inhibit eicosanoid synthesis, antiserum to PGE to specifically inhibit PGE, and norepinephrine to accelerate eicosanoid synthesis by stimulating release of free fatty acids including eicosanoid precursors.

Rats fed the LSO diet ate significantly more food than rats fed the HSO diet during the 31 feeding days ( $13.09 \pm 1.09$  g/day vs.  $14.23 \pm 0.36$  g/day, HSO vs. LSO). However, neither the mean body weights nor the mean weights of fat pads were different between the two diet groups.

Safflower oil contains approximately 74% linoleic acid, 13% oleic and palmitoleic acids, and 7% palmitic acid. Beef tallow contains 29% palmitic acid, 48% oleic acid, and 16% stearic acid (21). Therefore, the observed results show that the qualities of dietary fats were directly reflected in the fatty acid composition of epididymal fat pads.

This result is consistent with previous studies; dietary fatty acids were readily incorporated into the depot fat (61). The composition of fatty acids in the epididymal fat pads was also directly reflected in the composition of free fatty acids released from basal or norepinephrine-treated adipocytes during a 15-min incubation. The correlation coefficients ( $P < 0.05$ ) between per cent composition of individual fatty acids in the epididymal fat pads and per cent composition of the same fatty acids released from isolated adipocytes were +0.76, +0.92, +0.56, +0.99, and +0.78 for myristic, palmitic, stearic, oleic, linoleic, and linolenic acids. This result is different from the report of Christ and Nugteren (13) who reported that a catecholamine (epinephrine, 1  $\mu\text{g}/\text{mL}$ ) caused a specific release of linoleic acid, linolenic acid, and arachidonic acid compared with the other fatty acids from adipose tissue during 1 h incubation.

Aspirin (50 mg/Kg bw/day, 2 days) did not significantly alter concentrations or compositions of fatty acids in the epididymal fat pads compared with the controls. The aspirin feeding did not alter the composition of free fatty acids released from adipocytes compared with



the control, either. But the aspirin feeding significantly decreased concentrations of free fatty acids released from adipocytes (CON, 5,120.7  $\mu\text{g}/10^6$  cells, 1.8-times the ASP).

Isolated adipocytes from rats fed the HSO diet had significantly greater 2-DG transport during the 10-min incubation time compared with the LSO. The results suggest that the dietary linoleic acid content may modulate adipocyte glucose transport.

As shown in Figure 7, aspirin feeding did not significantly affect 2-DG transport into adipocytes during a 10-min incubation.

The observed, 2-DG transport values are in the range of previous reports. Olefsky (64) reported that in the basal state, 2-DG transport into isolated rat adipocytes was 7.3 nanomoles per min per  $10^6$  cells during a 3-min incubation at 24°C. Wieringa and Krans (93) reported that 2-DG transport into isolated adipocytes was 8.2 nanomoles/ $5 \times 10^5$  cells during a 3-min incubation in the basal state. In a review paper, Czech (18) claimed that the D-glucose transport system activity of fat cells showed a five-fold difference in apparent  $V_{\text{max}}$  of transport between two representative experiments and emphasized the variability between cell preparations. Significant variability in absolute values of adipocyte glucose transport and utilization among cell preparations is well known.

Norepinephrine, norepinephrine plus aspirin, or norepinephrine plus anti-PGE decreased 2-DG transport into adipocytes compared with the control in the HSO-CON group, but not in other groups. In the HSO-

CON group, the release of total free fatty acid was positively ( $P \leq 0.05$ ) correlated with 2-DG transport. Release of linoleic acid, arachidonic acid, and oleic acid was positively ( $P \leq 0.05$ ) correlated with 2-DG transport, too. But the release of linolenic acid was negatively ( $P \leq 0.05$ ) correlated with 2-DG transport. Among these free fatty acids, the effect of linoleic acid might be the greatest since the release of linoleic acid is in the greatest amount in the HSO-CON group.

The release of total free fatty acids was positively ( $P \leq 0.05$ ) correlated with 2-DG transport in the LSO-ASP group, too. Release of linoleic acid, oleic acid, stearic acid, and palmitic acid were also positively ( $P \leq 0.05$ ) correlated with 2-DG transport. Among these free fatty acids, the effect of oleic acid might be the greatest since the release of oleic acid is in the greatest amount in the LSO groups. As mentioned in "Review of Literature", reports about the effect of catecholamine on adipocyte glucose transport or metabolism are equivocal. Some reported positive effects (90, 74, 44, 45) and some reported negative effects (10, 79, 80, 93).

Aspirin treatment in vitro significantly decreased 2-DG transport into adipocytes compared with the controls in the HSO-CON group. The results suggest that endogenous eicosanoids may have a positive role in adipocyte glucose transport. The preliminary results in the present study showed that isolated rat adipocytes produced appreciable amounts of eicosanoids ( $PGE_1$ ,  $PGE_2$ ,  $PGI_2$  and  $PGF_{2\alpha}$  in a decreasing order for amounts of eicosanoids produced per unit number of cells). The

synthesis of PGE<sub>1</sub>, PGE<sub>2</sub>, and PGF<sub>2α</sub>, but not PGI<sub>2</sub> was increased by incubating cells with norepinephrine at 10<sup>-5</sup> M for 30 min. Aspirin treatment inhibits synthesis of group-2 eicosanoids. Adipocytes of the LSO group contained smaller eicosanoid precursor stores. Therefore, the ability of adipocytes of the HSO-ASP or the LSO-CON groups to synthesize eicosanoids would be much smaller than that of adipocytes of the HSO-CON group. This differential rate of eicosanoid synthesis might be the reason for the non-significant effect of aspirin treatment on 2-DG transport into isolated adipocytes in the HSO-ASP and the LSO-CON groups.

This result is consistent with several reports in which inhibitors of eicosanoids, such as indomethacin (2) and L8027 (53), inhibited glucose transport into isolated rat adipocytes. The results suggest that endogenous eicosanoids may have a positive role in adipocyte glucose transport.

On the other hand, aspirin plus norepinephrine in vitro significantly increased 2-DG transport into adipocytes compared with the control in the LSO-ASP group. Aspirin treatment itself did not significantly change 2-DG transport compared with the control. Adipocytes of the LSO-ASP group may have synthesized very little eicosanoids due to the effect of aspirin and the limited precursor availability. Therefore, the increment in 2-DG transport by aspirin plus norepinephrine may be attributed to the effect of norepinephrine rather than aspirin. Actually, the correlation coefficient between

total free fatty acids and 2-DG transport in the LSO-ASP group was positive ( $r=+0.58$ ,  $P=0.0115$ ,  $n=24$ ).

Anti-PGE significantly decreased 2-DG transport into isolated adipocytes in the HSO-CON group. The results suggest a positive effect of PGE on adipocyte glucose transport. On the other hand, anti-PGE did not change 2-DG transport in the HSO-ASP group or in the LSO groups which might be attributed to the low level of eicosanoid precursors available.

As mentioned in "Review of Literature", most in vitro experiments which used isolated fat cells resulted in a positive role of  $PGE_1$  (11, 35, 94) and  $PGE_2$  (2, 71) in glucose transport.  $PGF_{2\alpha}$  was reported to have a tendency of increasing glucose transport and glucose metabolism in isolated adipocytes but the results were not statistically significant (71).

The release of linoleic acids from isolated adipocytes was positively correlated ( $P\leq 0.05$ ) with 2-DG transport into adipocytes (HSO-CON,  $r=+0.56$ ; LSO-ASP,  $r=+0.74$ ;  $n=24$ ). The release of arachidonic acid was positively correlated ( $P\leq 0.05$ ) with 2-DG transport in the HSO-CON group ( $r=+0.63$ ;  $n=24$ ). These significant, positive correlations between precursors of eicosanoids and 2-DG transport suggest that eicosanoids have a positive role in adipocyte glucose transport. On the other hand, the release of linolenic acid was negatively ( $P\leq 0.05$ ) correlated with 2-DG transport (HSO-CON,  $r=-0.72$ ; HSO-ASP,  $r=-0.94$ ; LSO-ASP,  $r=-0.52$ ; LSO-ASP,  $r=-0.52$ ;  $n=24$ ). Alpha-linolenic acid or its

products inhibit synthesis of group-2-eicosanoids by competing with linoleic acid for  $\Delta^6$ -desaturase (4, 66). This inhibition of  $\alpha$ -linolenic acid on the synthesis of  $\gamma$ -linolenic acid might be the reason for the observed, negative correlations between the release of  $\alpha$ -linolenic acid and 2-DG transport.

The release of oleic acid, stearic acid, and myristic acid also were positively correlated ( $P \leq 0.05$ ) with 2-DG transport in certain diet groups (oleic acid in the HSO-CON and LSO-ASP groups, stearic acid in the ASP groups, myristic acid in the HSO-ASP group). The release of palmitic acid was positively correlated with 2-DG transport in the LSO-ASP group but negatively correlated in the HSO-ASP group. The mechanism of effects of these free fatty acids on 2-DG transport are not known.

In rat fat cells, a biphasic response of glucose transport to  $\beta$ -receptor agonist of catecholamines, such as isoproterenol, and other cAMP stimulators has been reported (45, 48). A low concentration of  $\beta$ -agonists stimulated basal glucose transport into adipocytes by increasing  $V_{max}$ , and high concentrations of  $\beta$ -agonists inhibited it by decreasing  $V_{max}$ . These results suggest that catecholamines and  $\beta$ -agonists may modulate glucose transport by affecting the glucose transporter.

## SUMMARY

1. A high PUFA (HSO) diet increased the content of eicosanoid precursors in epididymal fat pads compared with low PUFA (LSO) diet group.
2. The composition of fatty acids in the fat pad was directly reflected in the composition of free fatty acids released from isolated adipocytes.
3. HSO significantly increased glucose transport into isolated rat adipocytes compared with the LSO.
4. Aspirin feeding (ASP) did not significantly affect glucose transport into isolated rat adipocytes compared with the control group (CON).
5. Aspirin and anti-PGE in vitro significantly decreased glucose transport into rat adipocytes in the HSO-CON group compared with the controls.
6. Norepinephrine decreased glucose transport into isolated rat adipocytes in the HSO-CON group, and aspirin plus norepinephrine increased it in the LSO-ASP group compared with the controls.
7. The release of linoleic acid, arachidonic acid, oleic acid, and total free fatty acids was positively correlated with glucose transport into adipocytes.

## CONCLUSION

Dietary fatty acids affect the fatty acid composition of fat pad, and that affects adipocyte glucose transport. High PUFA diet increases adipocyte glucose transport while aspirin or anti-PGE decreasing it. These results suggest that the effect is via eicosanoids. PGE seems to be particularly involved.

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