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Forum Review

Factors Regulating Isoprostane Formation *In Vivo*

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ABSTRACT

Discovery of the F_2-isoprostanes, a group of prostaglandin $F_{2\alpha}$-like compounds biosynthesized from arachidonic acid nonenzymatically, has uncovered a new and novel facet of free radical biology. Some of these compounds are bioactive and thus may mediate adverse effects associated with oxidant stress. F_2-Isoprostanes have also been shown to be reliable biomarkers of lipid peroxidation. Factors influencing their formation and metabolism have been studied to some extent, although much remains to be determined. The purpose of this review is to summarize our current knowledge of conditions that modulate endogenous generation of these compounds. Isoprostanes have a wide daily variation in secretion in humans. Although normal levels can be defined, these compounds are found in increased concentrations in various pathophysiological states, including ischemia–reperfusion injury, atherosclerosis, and diabetes, and in experimental conditions of oxidative stress and inflammation. Alterations in isoprostane biosynthesis, secretion, and excretion in normal physiology and in pathophysiological states are due to the various types of endogenous and exogenous regulatory mechanisms that control the availability of precursors required for isoprostane synthesis, such as dietary and tissue arachidonic acid content, oxygen concentration, and the generation of various free radical species. Selected aspects of issues related to isoprostane formation and metabolism *in vivo* will be examined herein. *Antioxid. Redox Signal.* 7, 221–235.

INTRODUCTION

THE F_2-ISOPROSTANES, a group of prostaglandin F_{2a} ($PGF_{2\alpha}$)-like compounds, are biosynthesized from esterified arachidonic acid via a nonenzymatic free radical-catalyzed mechanism of lipid peroxidation *in vivo.* Some of these compounds have been shown to possess potent biological activity as pulmonary and renal vasoconstrictors and to modulate platelet activation. Several studies have shown that F_2-isoprostanes act as full or partial agonists of the G protein-coupled thromboxane receptor. In general, they have short half-lives. Following formation from esterified arachidonate, they are quickly hydrolyzed via various phospholipases and further metabolized by β-oxidation. A substantial portion of isoprostanes appear to undergo β-oxidation in tissues prior to release into the plasma. Intact isoprostanes, together with their β-oxidized metabolites, are efficiently excreted into the urine. Both clinical and experimental animal studies have shown increases in the formation of isoprostanes and their β-oxidation products in settings of acute and chronic inflammation, ischemia–reperfusion injury, diabetes, and atherosclerosis (8, 30, 90, 109). A number of reports have described that F_2-isoprostanes are reliable biomarkers of lipid peroxidation, and are useful *in vivo* indicators of oxidant stress in diverse conditions. They have also been used to assess the *in vivo* oxidative response to various drugs, antioxidants, or dietary interventions. As bioactive F_2-isoprostanes [mainly 8-iso-prostaglandin $F_{2\alpha}$(8-iso-PGF_{2a})] are continuously formed in various tissues under normal physiological conditions, and at elevated levels in various pathophysiological situations, endogenous and exogenous factors that regulate their formation are of clinical importance. This review summarizes the fac-

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tors that are currently known to control the biosynthesis, release, degradation, and excretion of F_2-isoprostanes *in vivo.*

OVERVIEW OF FACTORS REGULATING ENDOGENOUS ISOPROSTANE LEVELS IN TISSUES AND FLUIDS

The formation of F_2-isoprostanes requires arachidonic acid, molecular oxygen, and free radicals. Unlike cyclooxygenase (COX)-derived prostaglandins, isoprostanes have been shown to be formed primarily *in situ* from esterified arachidonate present in tissue phospholipids and to be released subsequently in the free acid form after hydrolysis of the ester moiety presumably by phospholipases (92). There are several points, at least theoretically, at which the formation of free isoprostanes might be regulated in tissues and biological fluids, including the initial oxidation step of arachidonate by free radicals, the deesterification process that leads to the formation of free isoprostanes, and the subsequent metabolism and excretion of both the parent compounds and degraded metabolites into the urine. Of these, the availability of molecular oxygen and free radicals in tissues appears to be the primary rate-limiting factor for isoprostane biosynthesis.

Following formation in tissues, esterified isoprostanes are enzymatically hydrolyzed *in situ* to form bioactive isoprostanes in their free acid form. This enzymatic cleavage step is also, at least theoretically, an important rate-limiting step for the formation of free isoprostanes in the circulation. Although not well studied, the availability of the hydrolytic enzymes such as phospholipase(s) A_2 in specific tissues or blood likely accounts for the release of free isoprostanes into the circulation. Free isoprostanes may also further undergo rapid metabolism via β-oxidation and efficient excretion into the urine. The presence of metabolizing enzymes is important for isoprostane degradation and subsequent excretion. A relative deficiency of hydrolytic and metabolizing enzymes could theoretically contribute to alterations in isoprostane levels *in vivo,* although to our knowledge, no studies have documented that endogenous levels of isoprostanes are greatly influenced by such deficiencies.

Numerous studies have shown that isoprostane levels are elevated in various human diseases and experimental conditions (8, 30, 64, 90, 109). Thus, levels of isoprostanes can be greatly influenced by pathophysiological situations. These include smoking, alcohol intake, exercise, drug treatment or various dietary antioxidant supplementation, and fruit and vegetable intake. Other exogenous factors that regulate isoprostane formation include various toxins and ischemia–reperfusion injury. It is generally assumed that excessive isoprostane production, rather than inefficient excretion or metabolism, accounts for elevated levels of these compounds in pathophysiological settings *in vivo,* although this has not been carefully studied. Other endogenous regulatory factors that may influence isoprostane formation include gender, age, ethnicity and pre- or postmenopausal state. These factors and their effects on endogenous isoprostane formation are discussed subsequently.

BIOSYNTHESIS AND METABOLISM OF ISOPROSTANES

The chemical mechanism of biosynthesis of isoprostanes from precursor arachidonic acid, which is an important factor in the regulation of isoprostane formation, is described in detail in several excellent reports and in this Forum issue, and thus will not be further discussed here (90, 91, 109).

Metabolism of isoprostanes has been shown to occur essentially via similar pathways as enzymatically formed primary prostaglandins. When radiolabeled 8-iso-$PGF_{2\alpha}$ (a major F_2-isoprostane) was infused over 2 h into a rhesus monkey, 95% of the radioactivity infused was excreted into the urine (111). Similarly, when radiolabeled 8-iso-$PGF_{2\alpha}$ was infused over l h into a male subject, 75% of the administered compound was excreted into the urine during the following 4.5 h (111). The half-life of 8-iso-$PGF_{2\alpha}$ has been found to be ~16 min in humans. The major excreted metabolite of 8-iso-$PGF_{2\alpha}$ was determined to be 2,3-dinor-5,6-dihydro-8-iso-$PGF_{2\alpha}$, which represented 29% of the total radioactivity. Thus, the major urinary metabolite of 8-iso-$PGF_{2\alpha}$ in humans is formed via one step of β-oxidation and reduction of the Δ^5-double bond (26, 111). Another major metabolite of 8-iso-$PGF_{2\alpha}$ in human and rat urine is 2,3-dinor-8-epi-$PGF_{2\alpha}$. In rat hepatocytes, metabolism of 8-iso-$PGF_{2\alpha}$ yields 2,3,4,5-tetranor-8-epi-PGF_{2a} (26).

In another metabolism study, when radiolabeled and unlabeled 8-iso-$PGF_{2\alpha}$ was administered intravenously to rabbits, the total radioactivity quickly disappeared from the circulation (6). About 80% of the total radioactivity was found in urine within 4 h in the rabbit as had been shown earlier in a human volunteer (111). The plasma half-life of 8-iso-$PGF_{2\alpha}$ in rabbits was found to be 1 min at the distribution phase (α-phase). The terminal elimination phase (β-phase) half-life was ~4 min. At 1.5 min after administration, 64%, 19%, and 13% of the plasma radioactivity represented 8-iso-$PGF_{2\alpha}$, l5-keto-$PGF_{2\alpha}$, and other β-oxidized products, respectively. The values for 20-min plasma were 5%, 2%, and 88%, respectively. Several of these polar β-oxidized metabolites found in the plasma were efficiently excreted into the urine. More polar β-oxidized products dominated the HPLC radio chromatograms of urine samples obtained at 10 min to 4 h after infusion. α-Tetranor-15-keto-13,l4-dihydro-8-iso-$PGF_{2\alpha}$ was identified as a major urinary metabolite in the rabbit along with several other β-oxidized products. The metabolism of 8-iso-$PGF_{2\alpha}$ to α-tetranor-15-keto-13,l4-dihydro-8-iso-$PGF_{2\alpha}$ and other β-oxidized products occurs via several degradation steps in the rabbit (6). A tentative metabolic pathway of 8-iso-$PGF_{2\alpha}$ in the rabbit is shown in Fig. 1.

In vitro studies with isolated rabbit tissues (4) and *in vivo* experiments in rabbits have shown that oxidation of the 15-hydroxy group of 8-iso-$PGF_{2\alpha}$ by 15-prostaglandin dehydrogenase (15-PGDH) is the first step of 8-iso-$PGF_{2\alpha}$ metabolism (Fig. 1) (6). A reduction of the C-13,14 double bond by Δ^{13}-reductase and formation of 15-keto-13,14-dihydro-8-iso-$PGF_{2\alpha}$ were shown to occur in the second step of metabolism. Thus, 15-PGDH and Δ^{13}-reductase are the major enzymes involved in the metabolism of 8-iso-$PGF_{2\alpha}$ in the rabbit. As various metabolizing enzymes, including phospholipases, hydrolyases, 15-PGDH, and Δ^{13}-reductase, are found in all rabbit

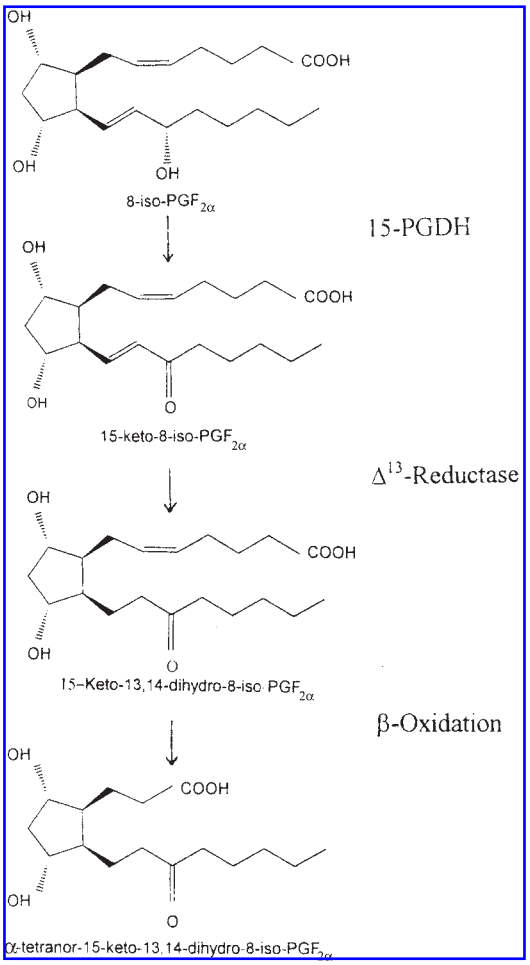

FIG. 1. Tentative metabolic pathway of 8-iso-$PGF_{2\alpha}$ in the rabbit. Reprinted with permission from Basu (6).

tissues (2, 12, 13, 92), it is likely that excessive formation of F_2-isoprostanes in any tissue would result in the formation of metabolites similar to those noted above.

In summary, these studies have provided insight into the metabolic pathways of one isoprostane, 8-iso-$PGF_{2\alpha}$, in animals and humans. Although metabolism varies to some extent, human, rabbit, and rat experiments confirm that β-oxidation is a common degradation pathway (Fig. 1).

INDIVIDUAL VARIATION OF ISOPROSTANE LEVELS IN HEALTHY HUMANS

The use of F_2-isoprostanes as a biomarker for lipid peroxidation *in vivo* in humans in different conditions and experimental settings requires knowledge as to how levels of this biomarker vary during a 24-h period and between days in healthy humans. Studies addressing these issues are surprisingly rare compared with the number of reports that have utilized F_2-isoprostanes as a biomarker of oxidative stress. This section summarizes studies related to the intraday and daily variation of 8-iso-$PGF_{2\alpha}$ in humans.

Variation within the day

For these studies, 10 healthy subjects collected a 24-h urine sample, a spot urine sample in the morning, and spot urine samples at varying hours during the day for measurement of 8-iso-$PGF_{2\alpha}$. As shown in Fig. 2, there is variation in isoprostane levels during the day. However, there was no significant difference at the group level comparing the mean values of 8-iso-$PGF_{2\alpha}$ in urine collected at different times during the day, or collected in the morning, compared with mean levels of 8-iso-$PGF_{2\alpha}$ in the 24-h urine sample ($p = 0.85$ and 0.69, respectively) (53). This was confirmed in a larger study with healthy individuals where the mean level of 8-iso-$PGF_{2\alpha}$ in the morning urine in the whole group did not differ from the mean level of 8-iso-$PGF_{2\alpha}$ in the 24-h collection (16). Further, no diurnal variation of 8-iso-$PGF_{2\alpha}$ at the group level could be observed when mean values from five different urinary collections were compared (106). No statistical circadian variation was observed on the group level between mean values of three 8-h collections during a 24-h period in 10 subjects (133). The individual values were not presented. Good correlations have been seen between levels of 8-iso-$PGF_{2\alpha}$ in the morning urine and levels of 8-iso-$PGF_{2\alpha}$ in the 24-h urinary collection, as well as in the spot urine collected at different times during the day and the 24-h urine, respectively (Fig. 3) (53). This was confirmed in the study by Richelle *et al.* where a good correlation between the levels of urinary 8-iso-$PGF_{2\alpha}$ collected in the morning or collected during 24 h is reported (106). Together, like other hormones and prostanoids, there is clearly a diurnal variation in levels of urinary 8-iso-$PGF_{2\alpha}$ during the day in each individual subject. On the other hand, when 8-iso-$PGF_{2\alpha}$ is evaluated on a group level, as in most clinical studies, the existing evidence agrees on a nonexisting circadian variation. Further, F_2-isoprostanes determined in the urine collected in the morning, or in several spot urine samples, adequately represent the daily F_2-isoprostane excretion.

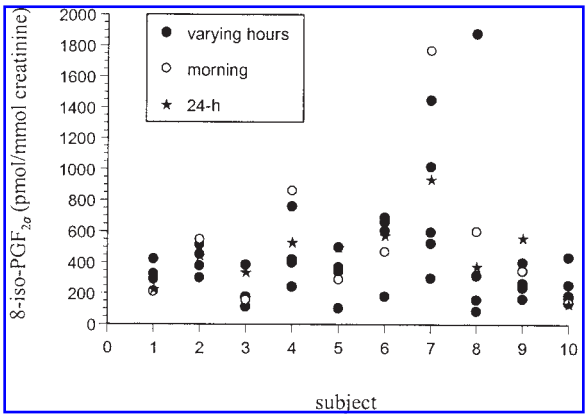

FIG. 2. 8-iso-$PGF_{2\alpha}$ in urine samples collected at various hours, in the morning, and during 24-h in 10 healthy subjects.

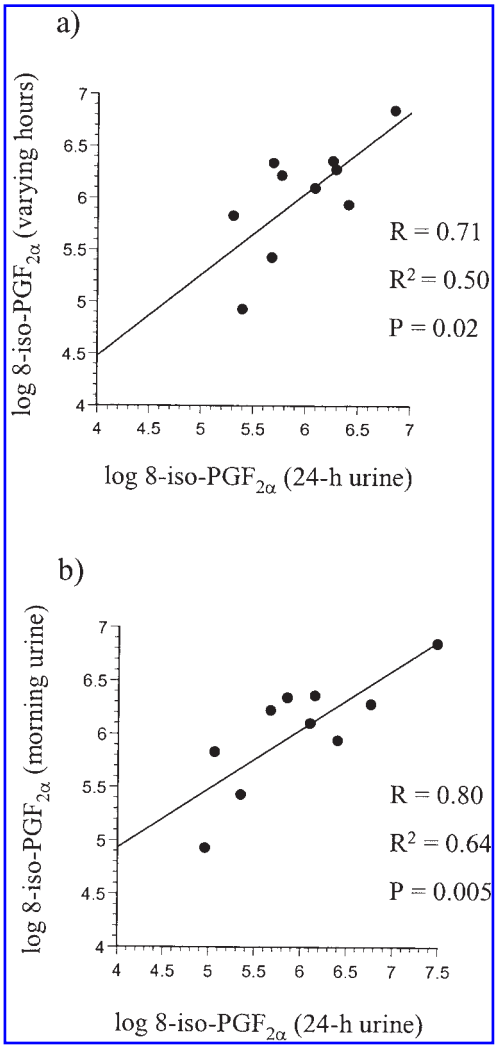

FIG. 3. Linear correlations of 8-iso-PGF$_{2\alpha}$ levels in (a) spot urine samples during a day (mean) and 24-h urine samples and (b) morning urine samples and 24-h urine samples, in 10 healthy subjects.

Variation between days

In a study we undertook, the intrasubject coefficient of variation in 8-iso-PGF$_{2\alpha}$ in morning urine during 10 consecutive days varied 18–104% in healthy subjects with a coefficient of variation of 42% for all 13 subjects during 10 days (54). This variation includes both a biological variation and an intraassay variation of the assayed samples. The variation due to the analysis technique used in this study has a coefficient of variation of ~12–15%, leaving the remaining observed variation (26–30%) to individual biological variation. The intrasubject variation during three different days varied 2.1–10.5% in nonsmokers and 4.5–24.3% in smokers (3). Individual variation between days is a factor to take into account when planning a clinical study, but the variation may vary depending on the setting.

OTHER ENDOGENOUS FACTORS AFFECTING THE ISOPROSTANE LEVELS

This section will discuss endogenous factors that affect the F_2-isoprostane formation. Both physiological factors, such as age, gender, ethnicity, and female hormones, and pathophysiological factors related to various diseases will be discussed. The diseases discussed in this review are restricted to metabolic disorders and risk factors for cardiovascular disease.

Physiological factors

The oxidative stress hypothesis of aging has led to extensive studies of the effect of age on isoprostane levels. Nonetheless, results are conflicting. A positive correlation between age and 8-iso-PGF$_{2\alpha}$ has been reported (129, 133), but older subjects had cardiovascular and metabolic diseases more frequently than did the younger subjects in the study. This finding might explain some of the positive correlation observed. In contrast, results from a Framingham cohort showed that 8-iso-PGF$_{2\alpha}$ was negatively correlated with age (69). One of the few studies of isoprostanes and children under the age of 7 years showed high 8-iso-PGF$_{2\alpha}$ levels in infancy that declined with age (67). Infections in early life might be a possible confounding factor in this study. However, most reports involving adults show a lack of relationship between age and 8-iso-PGF$_{2\alpha}$ levels (22, 46, 55, 83).

Gender is often a confounding factor in clinical studies. Female gender has been reported to be associated with higher levels of 8-iso-PGF$_{2\alpha}$ in some epidemiological reports (22, 69). These studies were large, allowing for multivariate analyses. It has also been reported that girls have higher 8-iso-PGF$_{2\alpha}$ levels than boys under the age of 7 (67). One study, however, has reported higher levels of 8-iso-PGF$_{2\alpha}$ in men than women (61), but this difference was driven by three men with high levels and, thus, the results cannot be extrapolated to the general population (31). Others have found a lack of association between isoprostanes and gender (83). Gender can modify the effect of a given drug or supplement on the formation of isoprostanes; male patients with hypercholesterolemia receiving vitamin E reduced their levels of 8-iso-PGF$_{2\alpha}$, whereas the female patients did not (113).

Ethnicity may also affect isoprostane formation in humans. Block *et al.* reported enhanced formation of 8-iso-PGF$_{2\alpha}$ in Caucasians compared with African Americans (22). Other studies have not found any difference between 8-iso-PGF$_{2\alpha}$ levels in Caucasians and African Americans at baseline (68, 77), but when acute hyperlipidemia was induced with infusion of fat, the formation of 8-iso-PGF$_{2\alpha}$ increased more in African Americans than in Caucasians (77).

Reports of the effect of female hormones on isoprostanes are few. Postmenopausal women had higher levels of 8-iso-PGF$_{2\alpha}$ than premenopausal women in a study of women with frequent migraine attacks (55). Pregnancy did not lead to altered levels of 8-iso-PGF$_{2\alpha}$ (89), whereas in another study, higher levels of 8-iso-PGF$_{2\alpha}$ were found among pregnant Japanese women compared with nonpregnant controls (19).

Metabolic diseases and cardiovascular risk factors

Obesity is a serious independent risk factor for cardiovascular disease. Women with a body mass index (BMI) of >28 have increased formation of 8-iso-$PGF_{2\alpha}$ regardless of the distribution of body fat (37). Patients with cardiovascular risk factors other than obesity had been carefully excluded from the study, suggesting that obesity is associated with enhanced oxidant stress. Loss of weight has been reported to result in a decrease in F_2-isoprostane levels (37). It has also been suggested that the lipolysis process during fasting might release 8-iso-$PGF_{2\alpha}$ from the adipose tissue, accounting for increased circulating levels of the compound seen during a 24-h fast (106). Other epidemiological studies of the association of weight and 8-iso-$PGF_{2\alpha}$ are conflicting: a positive correlation between 8-iso-$PGF_{2\alpha}$ and BMI or waist-to-hip ratio is described in some reports (22, 43, 69), but was not confirmed in a recent cohort of elderly men (56).

Patients with type 1 diabetes have been reported to have either unaltered formation (60, 130) or enhanced formation (35, 38) of 8-iso-$PGF_{2\alpha}$ compared with healthy subjects. Differences in the metabolic status (HbA1c and fasting glucose), degree of vascular disease, or hyperlipidemia could partly account for the conflicting results. Increased levels of 8-iso-$PGF_{2\alpha}$ formation were most pronounced in the early phase of the diabetes onset, and stabilized after 1 year in conjunction with the other metabolic parameters (38). Further, children with HLA-DR3/4 genotype, a high-risk genotype for type 1 diabetes, had higher levels of 8-iso-$PGF_{2\alpha}$ than the controls (67).

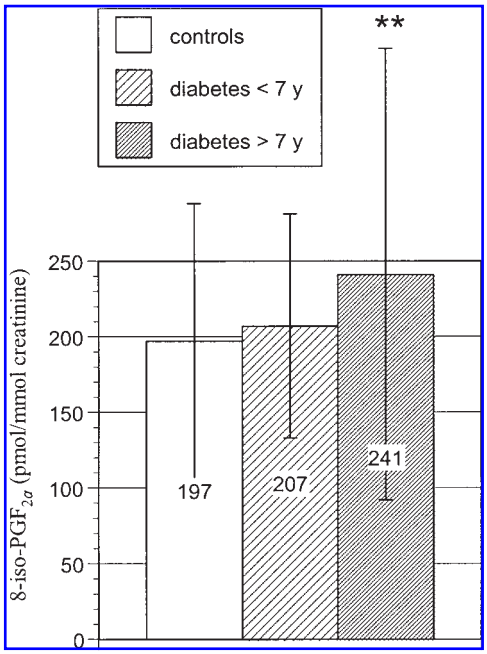

FIG. 4. 8-Iso-$PGF_{2\alpha}$ excretion in elderly men with type 2 diabetes with a disease duration less than 7 years, a disease duration of 7 years or more, and age- and sex-matched controls. Data are presented as means ± SD. $^{**}p < 0.01$ compared with controls.

Type 2 diabetes in humans is associated with higher levels of isoprostanes (35, 40, 49, 52, 56). Patients with impaired glucose tolerance (in a prediabetic state) had significantly higher levels of 8-iso-$PGF_{2\alpha}$ than healthy subjects with normal glucose tolerance (52). In contrast, a study of elderly men showed that only those who have had diabetes more than 7 years had elevated levels of 8-iso-$PGF_{2\alpha}$ (see Fig. 4) (56). Glucose and 8-iso-$PGF_{2\alpha}$ have been found to be positively correlated in some studies (35, 52), but not in others (49, 56). Induced hyperglycemia in patients with type 2 diabetes undergoing a glucose tolerance test has been reported to cause an acute elevation of 8-iso-$PGF_{2\alpha}$ formation (114).

Patients with mild-to-moderate essential hypertension have similar levels of 8-iso$PGF_{2\alpha}$ as normotensives (32, 76, 84). Hyperhomocysteinemia is associated with elevated levels of 8-iso-$PGF_{2\alpha}$, and plasma homocysteine is reported to be positively correlated with plasma and urinary 8-iso-$PGF_{2\alpha}$ (36, 131). High levels of serum cholesterol are related to increased formation of 8-iso-$PGF_{2\alpha}$ (34, 73, 99, 105). Serum cholesterol has been shown to be both positively correlated with 8-iso-$PGF_{2\alpha}$ (105) or not correlated (99).

EXOGENOUS FACTORS AFFECTING THE ISOPROSTANE LEVELS

Dietary fat and fatty acid supplementation

A high-fat Big Mac meal (Big Mac hamburger, French fries, and diet Coke) contains measurable amounts of 8-iso-$PGF_{2\alpha}$, but quantities are in the picomole range and unlikely to contribute to the plasma levels of 8-iso-$PGF_{2\alpha}$ if ingested (50). This has also been confirmed by an intervention study of nine subjects in which concentrations of 8-iso-$PGF_{2\alpha}$ in plasma were unaltered after intake of a Big Mac meal when the values were adjusted for plasma arachidonic acid (51). Formation of 8-iso-$PGF_{2\alpha}$ in four subjects was unchanged after 2 days of low-fat diet (only 5% fat as energy) (106), which is in line with the conclusion that 8-iso-$PGF_{2\alpha}$ is not easily affected by dietary lipid content *per se*.

Unfavorable dietary fat quality is an important risk factor to cardiovascular disease in humans. Certain polyunsaturated fatty acids (PUFAs) provide protection from cardiovascular diseases and, thus, a large number of clinical studies have been undertaken to study the *in vivo* effect of PUFAs on lipid peroxidation biomarkers, including isoprostanes. A change in fat quality from saturated fat to a rapeseed oil-based diet rich in α-linolenic acid (18:3, n-3) did not alter the formation and excretion of 8-iso-$PGF_{2\alpha}$ (120). However, when a diet consisting of saturated fat was replaced by a diet with high amounts of linoleic acid (18:2, n-6), levels of 8-iso-$PGF_{2\alpha}$ increased (124). A change from oleic acid to different amounts of the *trans* fatty acid vaccenic acid (18:2, *trans*-11) also increased 8-iso-$PGF_{2\alpha}$ formation (125). The difference in effect on 8-iso-$PGF_{2\alpha}$ in these studies could possibly be related to the number and placement of double bonds in the particular fatty acid being administered. Linoleic acid is a precursor of arachidonic acid, and thus may increase levels of 8-iso-$PGF_{2\alpha}$ by

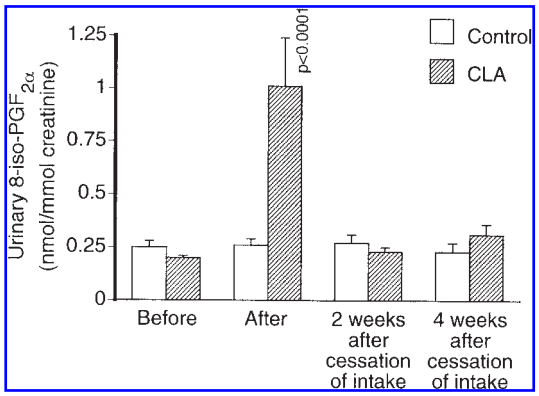

FIG. 5. Morning urinary levels of 8-iso-$PGF_{2\alpha}$ in control subjects and subjects treated with CLA for 1 month, and 2 and 4 weeks after the cessation of treatment. The *p* value indicates the significance for a difference between the changes in the two groups. Reprinted with permission from Basu *et al.* (15).

augmenting endogenous arachidonate synthesis. Vaccenic acid is converted to the conjugated linoleic acid (CLA) isomer 18:2 *cis*-9, *trans*-11 in humans (125), and this CLA isomer is known to result in elevation of levels of 8-iso-$PGF_{2\alpha}$.

The formation and excretion of 8-iso-$PGF_{2\alpha}$ increase remarkably when healthy subjects and men with the metabolic syndrome are supplemented with CLA (15, 16). Two weeks after cessation of intake of CLA, levels of 8-iso-$PGF_{2\alpha}$ returned to normal as shown in Fig. 5. The CLA isomer 18:2 *trans*-10, *cis*-12 is a significantly better inducer of 8-iso-$PGF_{2\alpha}$ formation than a mixture of the two isomers of CLA, namely, 18:2 *cis*-9, *trans*-11 and 18:2 *trans*-l0, *cis*-12 (107, 117).

Patients with type 2 diabetes given one fish meal daily reduced their 8-iso-$PGF_{2\alpha}$ levels by 20% (86). This result was confirmed in supplementation studies with long-chain PUFAs in fish, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Supplementation of EPA and DHA to healthy subjects, postmenopausal women, overweight hyperlipidemic men, or patients with type 2 diabetes resulted in reduced levels of 8-iso-$PGF_{2\alpha}$ compared with baseline levels (57, 87, 88, 97). However, in one study, the levels of 8-iso-$PGF_{2\alpha}$ were unaltered after EPA and DHA supplementation when adjusted for plasma arachidonic acid (57).

Other dietary factors affecting isoprostane formation

Fruits and vegetables are rich in antioxidants and could therefore inhibit isoprostane formation. Indeed, one epidemiological study has shown decreased levels of 8-iso-$PGF_{2\alpha}$ in humans who had the highest levels of fruit intake (22). Intervention studies have only, in part, confirmed this observation. A high amount of fruit and vegetables given to healthy humans for 4–6 weeks did not cause an alteration in the levels of 8-iso-$PGF_{2\alpha}$ (1, 45, 48, 76). A fruit- and vegetable-rich diet did, however, diminish the acute 8-iso-$PGF_{2\alpha}$ elevation caused by an experimental fat infusion (76). Further, supplementation with an antioxidant-rich vegetable burger and fruit concentrate for 3 weeks has been reported not to alter the levels of 8-iso-$PGF_{2\alpha}$ (128). Freshly squeezed orange juice (500 ml per day, 2 weeks) and aged garlic extract reduced the 8-iso-$PGF_{2\alpha}$ levels (44, 115). A diet rich in whole grain and legume powder reduced the 8-iso-$PGF_{2\alpha}$ levels in patients with coronary artery disease (63).

A recent intervention study using green tea extract or green or black tea did not alter 8-iso-$PGF_{2\alpha}$ levels in humans (47, 58). Further, a flavonoid-rich diet (enriched with onions and black tea) and chocolate with different amounts of procyanidin polyphenols did not alter levels of 8-iso-$PGF_{2\alpha}$ (101, 132). In contrast, dietary intervention with isoflavone phytoestrogens in soy decreased plasma 8-iso-$PGF_{2\alpha}$ formation in healthy individuals (134).

Vitamin E and vitamin C

Vitamin E (tocopherols, tocotrienols) is a lipid-soluble, chain-breaking antioxidant. Vitamin C is also an antioxidant, at least *in vitro,* and *in vivo* is involved in the recycling process of vitamin E. It might, therefore, be predicted that vitamins E and C, acting as antioxidants, would decrease 8-iso-$PGF_{2\alpha}$ formation *in vivo.* Several intervention studies have been reported in which either healthy subjects or those with various diseases were supplemented with vitamin E or vitamin C alone or in combinations. These studies are summarized in Table 1. In general, 8-iso-$PGF_{2\alpha}$ formation in healthy humans is not affected by different doses of α-tocopherol (82, 117). On the other hand, α-tocopherol supplementation has also been tested in patients where isoprostane formation is increased at baseline. A decrease in the elevated 8-iso-$PGF_{2\alpha}$ levels after vitamin E supplementation has been reported in humans with type 2 diabetes, hyperhomocysteinemia, and hypercholesterolemia (34–36). Further, isoprostane levels decreased in male patients with elevated serum cholesterol levels who were supplemented with moderate doses of α-tocopherol for 1–3 years (65, 113). More recently, F_2-isoprostane levels in humans with hypercholesterolemia were reduced only by very high doses of α-tocopherol, *e.g.*, 800 IU/day or more (110).

Isoprostane formation in smokers is not altered by vitamin E supplementation alone (102, 103). Vitamin C supplementation alone also does not affect 8-iso-$PGF_{2\alpha}$ formation in healthy young women, patients with type 2 diabetes, or volunteers with hypercholesterolemia (33, 65, 72, 113). On the other hand, supplementation with vitamin C decreased the levels of 8-iso-$PGF_{2\alpha}$ in heavy and overweight smokers (43, 103). Vitamin E and vitamin C theoretically potentiate each other's antioxidant effect, but placebo-controlled combination trials with vitamins E and C revealed no such effect on 8-iso-$PGF_{2\alpha}$ levels (29, 62, 65, 113). A combination of α-tocopherol and different carotenoids, on the other hand, decreased 8-iso-$PGF_{2\alpha}$ formation (127).

Smoking and alcohol

Cigarette smoking is associated with higher levels of 8-iso-$PGF_{2\alpha}$ in plasma, urine, breath condensate, and umbilical vessel tissue (3, 85, 94, 100, 102, 103). Increased formation of F_2-isoprostane levels has even been observed in children of smoking parents (116). Cessation of smoking reduced 8-iso-

TABLE 1. VITAMIN E AND VITAMIN C SUPPLEMENTATION AND EFFECTS ON 8-ISO-$PGF_{2\alpha}$ FORMATION

Supplementation (dose)	*Subject no./status*	*Study period*	*Result**	*Reference*	*Comments*
d-α-Tocopherol (200–2,000 IU/day)	25/healthy	8 weeks	→	82	Placebo-controlled
d-α-Tocopherol acetate (200 mg/day)	20/healthy	2 weeks	→	117	Placebo-controlled
dl-α-Tocopherol acetate (600 mg/day)	10/type 2 diabetes	2 weeks	↓	35	Controls missing
dl-α-Tocopherol acetate (100 or 600 mg/day)	22/high cholesterol	2 weeks	↓	34	Controls missing
d-α-Tocopherol (182 mg/day)	23/high cholesterol	12/36 months	↓	65, 113	ASAP study†, only men
d-α-Tocopherol (800 IU/day or higher)	35/high cholesterol	16 weeks	↓	110	Placebo-controlled
dl-α-Tocopherol acetate (300/600/1,200 mg/day)	11–12/smokers	3 weeks	→	102	Placebo-controlled
Vitamin E‡ (100 or 800 U/day)	7/smokers	5 days	→	103	Controls missing
dl-α-Tocopherol acetate (600 mg/day)	7/homocysteinemia	2 weeks	↓	36	Controls missing
Tocotrienols (200 mg/day)	17/high cholesterol	4 weeks	→	96	Mixed α- and γ-tocotrienol
Vitamin C (30–2,500 mg/day)	7/healthy		→	72	Young women only
Vitamin C (1,500 mg/day)	18/type 2 diabetes	3 weeks	→	33	Placebo-controlled
Vitamin C (500 mg/day)	25/high cholesterol	12/36 months	→	65, 113	ASAP study†
Vitamin C (2,000 mg/day)	5/heavy smokers	5 days	↓	103	Controls missing
Vitamin C (500 mg/day)	42/overweight smokers	60 days	↓	43	Placebo-controlled
α-TE§ (43 mg) + carotenoids (0.45 mg)	33/healthy	11 weeks	→	127	Supplemented in spreads
α-TE§ (111 mg) + carotenoids (1.24 mg)	33/healthy	11 weeks	↓	127	Supplemented in spreads
dl-α-Tocopherol (31 mg/day) Vitamin C (272 mg/day)	17/healthy+ 18/smokers	90 days	→	62	Placebo-controlled
d-α-Tocopherol (182 mg/day) + vitamin C (500 mg/day)	28/high cholesterol	12/36 months	→	65, 113	ASAP study†
Vitamin E‡ (800 U/day) + vitamin C (200 mg/day)	4/heavy smokers	5 days	↓	103	Controls missing
Vitamin E‡ (500 mg/day) + vitamin C (200 mg)	69/mild dementia	12 weeks	→	29	Placebo-controlled
Coenzyme Q10 (200 mg/day)	19/type 2 diabetes	12 weeks	→	59	Placebo-controlled

*→, F2-isoprostane formation unaltered compared with controls (or baseline); ↓, F2-isoprostane formation reduced compared with controls (or baseline).

†Antioxidant Supplementation in Atherosclerosis Prevention Study.

‡Type and preparation of vitamin E not specified.

§α-TE, α-tocopherol equivalents.

$PGF_{2\alpha}$ after 1 week (25, 103), and restarting smoking elevated levels again (25). Alcohol infusion into healthy subjects induces acute increases in the excretion of F_2-isoprostanes, and both acute alcoholic hepatitis and chronic liver cirrhosis are associated with elevated levels of F_2-isoprostanes (81).

Drugs

HMG CoA (3-hydroxy-3-methyl-glutarylcoenzyme A) reductase inhibitors (statins) are lipid-lowering drugs that protect against cardiovascular diseases. Further, they possess possible antiinflammatory properties. Treatment of humans with simvastatin, pravastatin, or cervastatin are associated with a reduction in F_2-isoprostane formation (39, 42, 71, 123). Other lipid-lowering drugs (fenofibrate, bezafibrate) have no effect on formation of 8-iso-$PGF_{2\alpha}$ (42, 59).

Because isoprostanes are hypothesized to be formed independent of COX, a number of studies have examined the effect of nonsteroidal antiinflammatory drugs (NSAIDs) on 8-iso-$PGF_{2\alpha}$ in humans. Aspirin, ibuprofen, naproxen, and indomethacin do not affect F_2-isoprostane formation in healthy subjects (24, 91, 133). Further, aspirin, indobufen, and indomethacin given to patients with increased formation of 8-iso-$PGF_{2\alpha}$ (such as patients with diabetes or hypercholesterolemia or smokers) did not alter the level of 8-iso-$PGF_{2\alpha}$ (34, 35, 49, 103). Rofecoxib (a COX-2 inhibitor) given to healthy subjects did not alter the levels of F_2-isoprostanes in a placebo-controlled study (117). Thus, work performed to date

shows that 8-iso-$PGF_{2\alpha}$ formation is not affected by treatment with NSAIDs.

Estrogen and progestin hormone therapy in postmenopausal women has been reported to decrease the formation of 8-iso-$PGF_{2\alpha}$ (68). Further, oral treatment with raxofelast (a vitamin E-like antioxidant) reduces 8-iso-$PGF_{2\alpha}$ levels in patients with type 2 diabetes (28). Finally, angiotensin II infusion induces F_2-isoprostanes in human hypertensives during high saltintake (95).

Other exogenous factors

8-Iso-$PGF_{2\alpha}$ formation in astronauts on the Russian space station MIR has been studied during and after prolonged space flight. Levels were decreased during the flight and increased post-flight compared with pre-flight (122). The authors explain that the changes in excretion of 8-iso-$PGF_{2\alpha}$ are a consequence of a deficit in energy intake in-flight and compensatory increased metabolic activity post-flight. Radiotherapy to the prostate gland, as treatment for prostate cancer, does not increase urinary F_2-isoprostane formation (23).

Prolonged exercise training is associated with increases in plasma 8-iso-$PGF_{2\alpha}$ in humans during the last part of the training and 1 h after training (78, 98, 121). Eccentric exercise, leading to muscle damage and inflammation, is also associated with an increased level of F_2-isoprostanes a few days after exercise (27, 112). Moderate or light training three times a week for 3 weeks did not alter 8-iso-$PGF_{2\alpha}$ levels in patients with type 2 diabetes (86), whereas patients with various risk factors for cardiovascular disease on a low-fat high-fiber diet who performed a daily walking tour for 3 weeks decreased their 8-iso-$PGF_{2\alpha}$ formation (108).

Human reperfusion injury

Numerous studies have shown that free radical-mediated oxidative stress and lipid peroxidation may play a major role in the pathophysiological sequelae of ischemia–reperfusion injury (79, 80). Ischemia–reperfusion injury is a common feature in patients undergoing thrombolysis, cardiopulmonary bypass, thoracoabdominal aortic replacement, organ transplantation, cardiac arrest, and stroke. Plasma levels of 8-iso-$PGF_{2\alpha}$ have been shown to increase early in patients undergoing percutaneous transluminal coronary angioplasty following acute myocardial infarction, and increased levels returned to normal within 4 h (70). Further, in a recent study, plasma levels of 8-iso-$PGF_{2\alpha}$ increased immediately in the patients undergoing elective percutaneous coronary intervention and coronary angiography (21). The levels returned to normal within 3–4 h. Urinary levels of 8-iso-$PGF_{2\alpha}$ were elevated in patients with acute myocardial infarction given thrombolytic therapy compared with the age-matched control subjects (41, 104). Also, plasma levels of 8-iso-$PGF_{2\alpha}$ increased significantly within 3 min and continued until 50 min during cardiopulmonary bypass (CPB) in patients with diverse coronary artery diseases (126). Levels returned to normal at 6 h after the CPB, and remained at baseline until 24 h. The mean plasma levels of 8-iso-$PGF_{2\alpha}$ before, during, and following CPB are shown in Fig. 6.

In conclusion, a number of studies have reported that F_2-isoprostanes increase during ischemia–reperfusion injury, supporting a potential role for these compounds in the pathological sequelae of this entity (66).

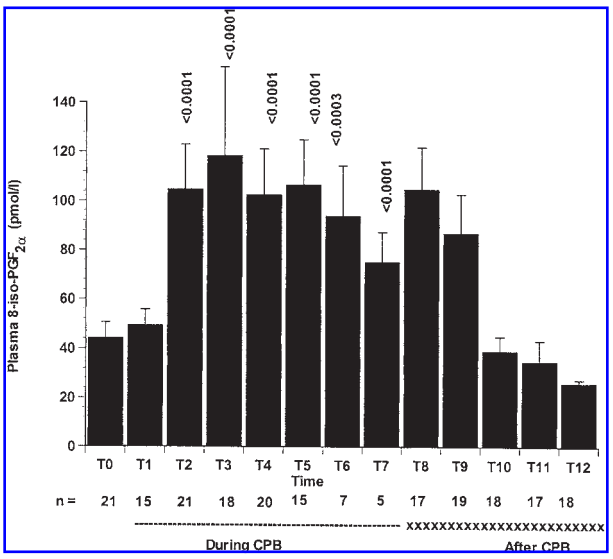

FIG. 6. Levels of free-8-iso-$PGF_{2\alpha}$ in peripheral plasma at various times before, during, and following CPB. T0, baseline, preinduction from anesthesia; T1, post intubation; T2, 3 min after CPB; T3, T4, T5, T6, and T7, 10, 20, 30, 40, and 50 min after CPB; T8, end of CPB; T9, after protamin infusion; T10, T11, T12, 6, 12, and 24 h after CPB. Reprinted with permission from Ulus *et al.* (126).

ISOPROSTANES IN EXPERIMENTAL ANIMAL MODELS

Further insights into factors regulating isoprostane formation *in vivo* have emerged from studies utilizing animal models of oxidant stress. We briefly summarize a portion of this work that is of relevance to human physiology and pathophysiology.

Models of oxidant injury in animals

A large number of experimental animal models of oxidant stress have been reported to be associated with an increased formation of isoprostanes (7, 8, 93, 119). Carbon tetrachloride (CCl_4) is a well known hepatotoxin that induces free radical injury in the liver and other tissues when administered to rats. Administration of this agent has been shown to result in large increases in esterified isoprostanes in liver tissue within 1–2 h of treatment (7, 93, 118). Significant increases in free 8-iso-$PGF_{2\alpha}$ can also be detected in rodent plasma and urine 2–6 h after oral administration of CCl_4 (5, 119). In another study in rats, it was shown that the levels of free 8-iso-$PGF_{2\alpha}$ were increased l7-fold in the plasma and 53-fold in the urine at 4 h compared with the basal levels after oral administration of $CC1_4$ (7). Levels of F_2-isoprostanes were still significantly higher at 24 and 48 h after the administration of $CC1_4$ as compared with the baseline values (93). The biosynthesis and release of isoprostanes were proportional to the dose of

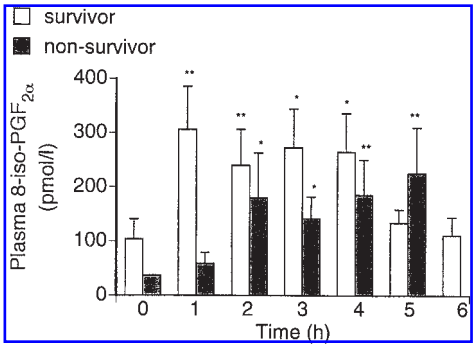

FIG. 7. Mean levels of 8-iso-$PGF_{2\alpha}$ in plasma in surviving and nonsurviving endotoxemic pigs. *$p < 0.05$; **$p < 0.01$. Reprinted with permission from Basu and Eriksson (9).

CCl_4. These results indicate that isoprostane formation can be increased by hepatotoxic substances that induce lipid peroxidation, and provide a useful model to test the effects of antioxidants on endogenous isoprostane generation (93, 109, 119).

Septic shock model

Human gram-negative septic shock is a frequent condition in intensive care units and is associated with significant mortality. This acute inflammatory event can be replicated experimentally in pigs administered *E. coli* lipopolysaccharide. Oxidant stress is presumed to be associated with septic shock. Both plasma and urinary levels of 8-iso-$PGF_{2\alpha}$ increased dramatically in a well established porcine model of septic shock following intravenous administration of *E. coli* lipopolysaccharide in conjunction with COX-mediated $PGF_{2\alpha}$ formation (9–11). Thus, induction of acute inflammation by endotoxemic challenge is associated with increased lipid peroxidation and F_2-isoprostane formation (Fig. 7). Further, the survival of pigs was dependent on the kinetics of formation and the levels of 8-iso-$PGF_{2\alpha}$ in the circulation, and there was an increase in arterial partial pressure of CO_2 among nonsurvivors compared with the survivors (9, 10). An inverse relationship was also seen between plasma levels of 8-iso-$PGF_{2\alpha}$ and vitamin E in this experimental model (11). Propofol (Diprivan-EDTA), an anesthetic and sedative agent with antioxidative properties, reduced endotoxin-induced plasma F_2-

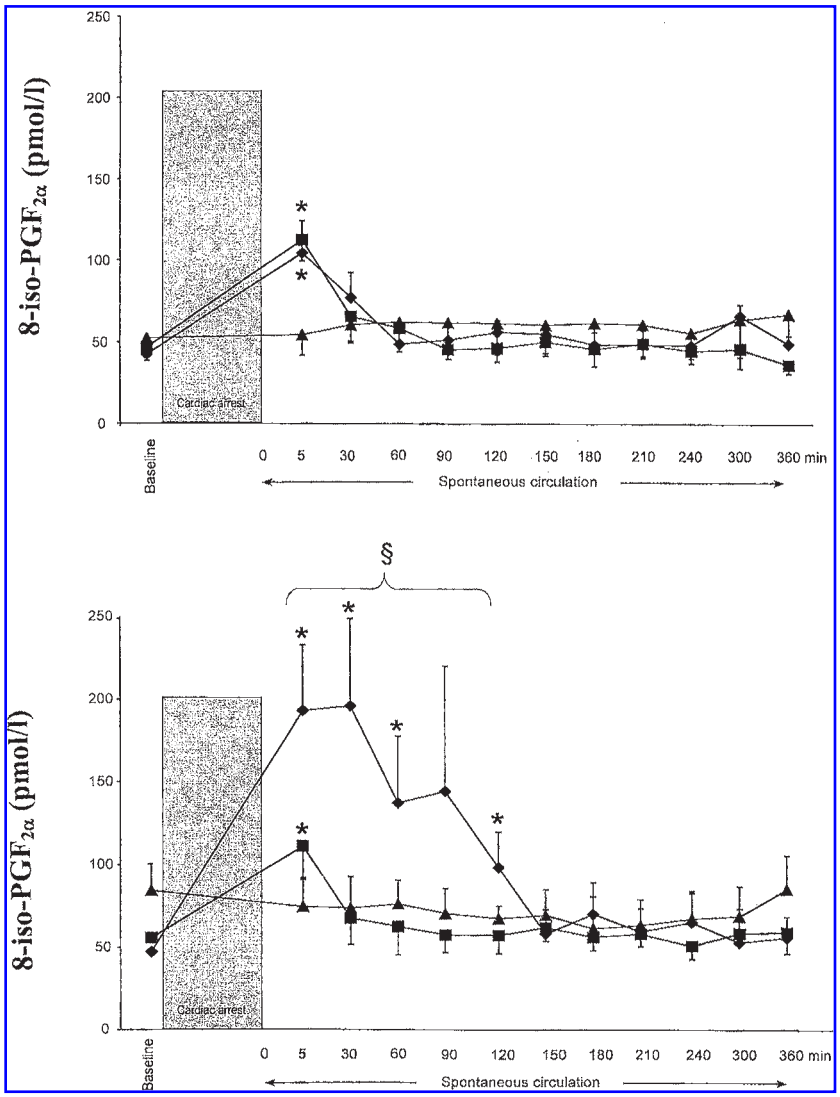

FIG. 8. Mixed venous plasma levels of 8-iso-$PGF_{2\alpha}$ at baseline and after ROSC (upper panel) and jugular bulb plasma levels of 8-iso-$PGF_{2\alpha}$ at baseline and after ROSC (lower panel). ◆, group VF5; ■, group VF2; and ▲, control group. *Significant difference versus baseline; §, significant difference between group VF5 versus other groups. Values are expressed as means ± SEM. Reprinted with permission from Basu *et al.* (14).

isoprostanes to the basal levels and diminished the fall in arterial oxygen tension (18). Thus, the increased lipid peroxidation and isoprostane formation seems to be associated with acute inflammatory response mediated-free radical formation through septic shock and subsequent organ dysfunction.

Cardiac arrest and brain damage model

Ischemia–reperfusion is tightly associated with experimental cardiac arrest and subsequent cerebral injury. It is well known that reperfusion causes local and remote organ damage that is a severe and frequently observed clinical manifestation. In an experimental porcine model of cardiopulmonary resuscitation, oxidative injury was assessed by the measurement of 8-iso-$PGF_{2\alpha}$ in plasma samples collected both from systemic circulation and from the jugular bulb, which drains the brain (14). 8-Iso-$PGF_{2\alpha}$ increased rapidly both in the systemic circulation (Fig. 8, upper panel) and in jugular bulb plasma (Fig. 8, lower panel) after cardiac arrest and resuscitation, concomitant with increases in the levels of a COX-mediated $PGF_{2\alpha}$ metabolite, hypoxanthine, and lactate (14, 74, 75). Recently, it was also shown that a time-dependent increase in jugular bulb plasma 8-iso-$PGF_{2\alpha}$ formation followed ventricular fibrillation of various duration (2, 5, 8, 10, and 12 min) and CPR (5 and 8 min) (20). Interestingly, the neurological outcome 24 h after experimental CPR was shown to correlate with cerebral plasma 8-iso-$PGF_{2\alpha}$ concentrations after restoration of spontaneous circulation (ROSC) (74). Increased production of 8-iso-$PGF_{2\alpha}$ has also been observed in acute coronary thrombolysis/reperfusion in an experimental canine model (41). Further, rapid appearance of 8-iso-$PGF_{2\alpha}$ in the plasma and urine was observed during ischemia–reperfusion in experimental spinal cord ischemia in pigs (17). Taken together, these studies support the concept that isoprostanes not only increase in models of ischemia–reperfusion but may also play a role in the pathophysiological sequelae of this injury.

CONCLUDING REMARKS

A number of regulatory mechanisms control isoprostane formation *in vivo.* These can impact the formation of isoprostanes or modulate their metabolic disposition. Ultimately, the formation of these compounds is dependent on the availability of arachidonic acid, molecular oxygen, and free radicals. Isoprostane formation, however, can be influenced by a number of endogenous and exogenous factors that have been discussed herein. For example, 8-iso-$PGF_{2\alpha}$ levels vary both within the day and on a day-to-day basis in individual humans, but this variation diminishes when large numbers of humans are studied as a group. A pattern in circadian variation of isoprostane formation has not been found, and a morning urine sample (or several spot urine samples collected during the day) adequately reflects the diurnal excretion of 8-iso-$PGF_{2\alpha}$. Factors such as age, gender, ethnicity, and several cardiovascular risk factors (type 2 diabetes, obesity, hypercholesterolemia, and smoking) are associated with altered 8-iso-PGF_{2a} formation. Dietary fat content can, in some cases, also alter 8-iso-$PGF_{2\alpha}$ generation. Fruits, vegetables, and tea do not generally change basal 8-iso-$PGF_{2\alpha}$ formation. Supplementation with vitamin E or vitamin C does not alter baseline levels of isoprostanes in healthy humans, but vitamin E supplementation may decrease elevated levels of 8-iso-$PGF_{2\alpha}$ in particular experimental settings. Drugs such as statins reduce 8-iso$PGF_{2\alpha}$ formation, whereas COX-l and COX-2 inhibitors do not have any effect. Further, strenuous exercise may increase formation of isoprostanes.

It is clear, therefore, that the generation of isoprostanes is regulated by many factors. Work over the past decade has provided important insights into how levels of these compounds in human biological fluids and tissues are controlled. It is predicted that ongoing and future studies will provide additional important information about the formation and metabolism of these bioactive lipid markers.

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ABBREVIATIONS

BMI, body mass index; CCl_4, carbon tetrachloride; CLA, conjugated linoleic acid; COX, cyclooxygenase; CPB, cardiopulmonary bypass; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; 8-iso-$PGF_{2\alpha}$, 8-iso-prostaglandin $F_{2\alpha}$; NSAID, nonsteroidal antiinflammatory drugs; 15-PGDH, 15-prostaglandin dehydrogenase; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PUFA, polyunsaturated fatty acid; ROSC, restoration of spontaneous circulation.

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