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Metabolism Clinical and Experimental 55 (2006) 119–127

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Skeletal muscle and liver oxysterols during fasting and alcohol exposure

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Received 27 March 2004; accepted 2 August 2005

Abstract

Oxysterols are cytotoxic agents that have a range of cellular actions, including impairment of albumin synthesis, cell differentiation, and induction of apoptosis. Their regulations by nutritional factors are poorly described. Our objective was to test the hypothesis that the imposition of food withdrawal and alcohol exposure increases tissue oxysterol concentrations. We measured the concentrations of the oxysterols 7α-hydroxycholest-5-en-3β-ol (7α-OH), 7β-hydroxycholest-5-en-3β-ol (7β-OH), and 3β-hydroxycholest-5-en-7-one (7-keto) in liver and skeletal muscle of fed and fasted (food withdrawal for 1 and 2 days) male Wistar rats. Both oxidative (type I; soleus) and glycolytic (type II; plantaris) muscles were analyzed. We also investigated the effects of a nutritional perturbant induced by a short-term bolus of ethanol (75 mmol/kg weight IP administered 2.5 hours before sacrifice). The results showed that in response to fasting there were significant increases in 7α-OH, 7β-OH, and 7-keto in liver and both type I and II skeletal muscle ($P < .001$ in all instances). For skeletal muscle, the increases were blunted or ameliorated after 2 days when compared with data from rats starved for 1 day. In contrast, the increases in liver after 1 day's fasting were relatively sustained at 2 days. Short-term ethanol increased 7α-OH, 7β-OH, and 7-keto in type I muscle of fed animals only ($P < .001$ in all instances) with a significant interaction between fasting and alcohol ($P < .001$ in all instances). For the first time, we have shown that oxysterols can increase in muscle and liver in response to food withdrawal and in response to an immediately imposed nutritional perturbant (ie, alcohol). Increased oxysterols represent elevated oxidative stress and/or disturbances in their formation or clearance. Because of the reported cytotoxic properties of oxysterols, these data are important in understanding cellular pathology because episodic anorexia and/or oxidative stress occur in a variety of disease conditions including sepsis, cancer cachexia, ischemia, and hormonal imbalance.

1. Introduction

Oxysterols are derived from cholesterol via free radical/reactive oxygen species activity or via oxidation (Fig. 1). The membrane domain of the cell and subcellular organelles are rich in cholesterol [1,2] and particularly sensitive to free radical–mediated damage [2,3].

Oxysterols are cytotoxic agents, acting at both the cellular and molecular levels and affecting a range of metabolic processes including impairment of albumin synthesis [4], induction of cytokine production [5], cell differentiation [6], gap junction dysfunction [7], and apoptosis [8-11]. For example, oxysterols elevate involucrin and transglutaminase 1 messenger RNA and protein concentrations of human keratinocytes [6], and Bcl2 protein decreases in smooth muscle cells exposed to oxysterols [12]. It has been argued that oxysterols are the most toxic form of oxidized lipid in low-density lipoprotein [13].

We have shown that the cholesterol-derived markers are particularly sensitive markers of oxidative stress compared with other convention markers such as protein carbonyls [14-16]. In previous studies, we showed that long-term alcohol feeding increased oxysterols in muscle [17] and liver [18]. This could have been because of either the imposition of malnutrition (eg, malabsorption is well known to occur in alcoholism), reactive oxygen species acting on

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doi:10.1016/j.metabol.2005.08.003

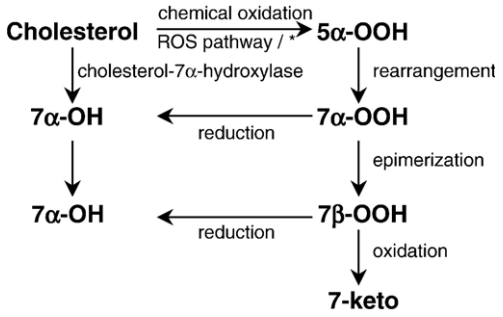

Fig. 1. Oxysterols in mammalian tissues. Asterisk indicates artificially increase in processing steps; 5α-OOH, 5α-hydroperoxycholesterol; 7α-OOH, 7α-hydroperoxycholesterol; 7*β*-OOH, 7*β*-hydroperoxycholesterol; ROS, reactive oxygen species.

the membrane domain, or a consequence of one of the many metabolites arising from alcohol metabolism (eg, acetaldehyde). However, although there is a considerable amount of information on the cellular pathoactivity of oxysterols, the effects of nutritional status on the concentrations of oxysterols are unknown.

We hypothesized that the concentrations of oxysterols increase in catabolic disorders such as those induced by food deprivation and cytotoxic reactions or metabolic perturbations induced by noxious agents and/or oxidative stress.

To test this hypothesis, we measured the concentrations of 3 closely linked oxysterols, namely, 7α-hydroxycholest-5-en-3β-ol (7α-OH), 7β-hydroxycholest-5-en-3β-ol (7β-OH), and 3*β*-hydroxycholest-5-en-7-one (7-keto) in liver and skeletal muscle of fed and fasted rats. We also investigated the effects of an additional nutritional perturbation induced by a short-term bolus of ethanol. This is of particular relevance because a number of studies have shown that imposition of alcohol with a preexisting stress exaggerates the effects of the single insult (ie, the concept of metabolic superimposition). We argue that models in which there is a single imposition may be classified as artificial or limiting, because in the clinical scenario, a single disease will be characterized by a number of concomitant cellular or physiological disturbances.

2. Materials and methods

2.1. Materials

3,5-Di-*tert*-butyl-4-hydroxytoluene, luminol (3-aminophthaloylhydrazine), and cytochrome *c* (from horse heart, type VI) were purchased from Wako Pure Chemical (Osaka, Japan). 7-Keto, 7α-OH, 7β-OH, and β-sitosterol (an internal standard for high-performance liquid chromatography [HPLC]) were purchased from Steraloids (Wilton, NH). Ethanol and saline (0.15 mol/L NaCl) for injection were obtained from King's College Hospital Pharmacy (London, UK; >99% purity). The ethanol solution was made up by mixing 54.6 mL ethanol with 0.15 mol/L NaCl to 100 mL. All other chemicals were of optimum purity (7β-OH, >95%; 7-keto, >90%; β-sitosterol, >99.5%) and of analytical grade.

2.2. Animal treatments

Male Wistar rats were obtained from accredited commercial suppliers at about 60 g body weight. They were maintained in a temperature- and humidity-controlled animal house for approximately 1 week until they weighed approximately 0.1 kg. They were then ranked and divided into 6 groups of equal mean body weight, and 2 groups had their food withdrawal for either 1 or 2 days. All doses of saline or ethanol were injected intraperitoneally. Thus, at 2.5 hours before sacrifice, rats were injected with either saline (0.15 mol/L NaCl; 10 mL/kg body weight IP) or ethanol (75 mmol/kg body weight; 10 mL/kg body weight IP) as described previously [19]. Groups were thus (A) fed + NaCl; (B) fed + EtOH; (C) fasted 1 day + NaCl; (D) fasted 1 day + EtOH; (E) fasted 2 days + NaCl; and (F) fasted 2 days + EtOH. Animals were killed by decapitation. Liver (portions of about 200-300 mg) and oxidative (type I; soleus) and glycolytic (type II; plantaris) muscles were rapidly dissected for subsequent analysis. The work had been carried out in the UK under institutional supervision that ensured humane treatment of the animals. Some of the tissues harvested in these experiments have been used by other groups in other studies not related to muscle or liver oxysterols so as to minimize animal usage in accordance with home office guidelines.

2.3. Tissue extraction procedures for lipids

Total lipid was extracted by adding 4 mL of ice-cold chloroform/methanol (3:1, vol/vol), containing 0.005% (vol/vol) butylated hydroxytoluene (as antioxidant), and 60 nmol *β*-sitosterol as an internal standard for HPLC-UV, to approximately 0.1 g of tissue and homogenized in a Polytron motor-driven tissue disrupter under ice-cold conditions. The homogenate was mixed with 4 mL of chloroform/methanol (3:1, vol/vol) and 1 mL of distilled water, vortexed vigorously, and centrifuged (800*g*). The chloroform layer was aspirated, concentrated in a rotary evaporator, and dried under nitrogen. A cholesterol-rich fraction was isolated from the total lipid by solid-phase extraction. A silica column (Sep-Pak, Waters Milford, MA) packed with aminopropyl-derivatized silica ($-NH_2$) was initially conditioned by washing with 5 mL of acetone and 10 mL of *n*-hexane. The total lipid sample, dissolved in a small amount of chloroform, was added to the column, which was flushed with a mixture of 2 mL chloroform and 1 mL isopropanol, giving an eluate that mainly consisted of cholesterol. This was concentrated in a rotary evaporator and dried under a nitrogen stream. The residue was dissolved in methanol and stored at 4°C until analysis.

2.4. High-performance liquid chromatography–UV analysis of oxysterols

Oxysterols were determined by HPLC using equipment that was composed of a L-7100 pump (Hitachi, Tokyo, Japan), SPD-10Avp UV detector (Shimadzu, Japan) set at

210 and 245 nm, and a Chromatopac C-R8A integrator (Shimadzu, Japan). An Inertsil ODS-2 column (GL Sciences, Tokyo, Japan) was used (5 μm, 150 × 4.6 mm internal diameter), and acetonitrile/methanol/water (46:45:9) was used as the mobile phase at the flow rate of 0.7 mL/min. All oxysterols were detected at 210 nm, whereas 7-keto was detected at 245 as well as 210 nm. The area of absorbance at 245 nm was 2.6 times as large as at 210 nm (hence, the determination of 7-keto at 245 nm). Standard curves were prepared by the analyses of 25 to 200 ng of 7α-OH, 50 to 200 ng of 7β-OH, and 7-keto using 250 ng of internal standard (β-sitosterol).

Theoretically, the data for oxysterols can be normalized in terms of dry weight, wet weight, DNA (reflecting the number of cells), total organ content, total lipid, or cholesterol. However, we present the data in terms of wet weight for consistency with our previous work in this field. Presenting data in terms of wet weight effectively portrays the relative abundance of oxysterols within the cellular and cytoplasmic milieu.

2.5. Statistical analysis

The data are expressed as mean ± SEM (5-8 observations in each group) nanomoles of analyte per gram of wet tissue weight and were analyzed using between-subjects 2 × 3 two-way analysis of variance (ANOVA), with treatment (ethanol, no ethanol) and fasting status (no fasting, fasting 1 day, fasting 2 days) as independent factors. Significance was indicated when $P < .05$ or $P = .05$. Posterior statistical analysis was conducted to unpack statistically significant main effects or higher-order interactions observed using the least significant difference test and simple effects analysis. Posterior analysis was conducted only after close visual scrutiny of graphically represented data to reduce the number of post hoc comparisons made to a minimum, to protect the data analysis from an elevated risk of type I statistical error [20].

3. Results

As reported previously [17], high-performance liquid chromatographic separation of standard 7α-OH, 7β-OH, and 7-keto, and the internal standard β-sitosterol was successful (Fig. 2). The retention times of all 3 oxysterols in tissue samples were identical to standard solutions (Fig. 2). In the following sections, we describe the responses of the soleus, plantaris, and liver to ethanol and fasting. The apparent differences in the responses of the 2 muscle types are further rationalized in the Discussion.

3.1. Effects on 7α-OH

3.1.1. Soleus

ANOVA showed that both fasting ($P < .001$) and ethanol treatment ($P < .001$) increased 7α-OH. A highly significant interaction ($P < .005$) was observed between fasting and ethanol treatment. Post hoc pairwise comparisons revealed

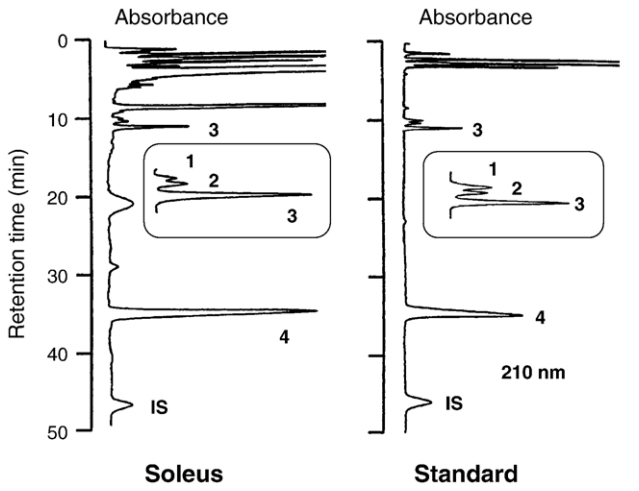

Fig. 2. Analytical detection and measurement of oxysterols in muscle. High-performance liquid chromatography analysis of standard cholesterol oxidation products with UV detection at 210 nm. Right, standard solutions; left; representative trace from rat tissue (ie, soleus muscle). Peak 1 indicates 7α-OH; peak 2, 7β-OH; peak 3, 7-keto; peak 4, cholesterol; 210 nm, the wavelength used for detection.

1-day–fasting 7α-OH concentrations to be significantly higher than 2-day fasting ($P < .005$) and fed ($P < .001$) observation points. Two-day fasting 7α-OH concentrations were significantly higher than fed ($P < .005$) levels. Simple effects analysis revealed ethanol increased soleus 7α-OH in the fed animals ($P < .001$; Fig. 3).

3.1.2. Plantaris

ANOVA showed that fasting increased 7α-OH concentrations in plantaris ($P < .001$). Post hoc analysis showed that concentrations in plantaris at 1-day fasting were significantly higher than concentrations seen in fed or at 2-day fasting animals ($P < .001$ in both instances; Fig. 3).

3.1.3. Liver

ANOVA showed that a highly significant main effect of fasting ($P < .001$) was observed. Post hoc pairwise comparisons revealed 1-day fasting 7α-OH concentrations to be significantly higher than at the fed ($P < .001$) observation point. Furthermore, 2-day 7α-OH concentrations were observed to be significantly higher than at the fed ($P < .001$) observation point (Fig. 3).

3.2. Effects on 7β-OH

3.2.1. Soleus

ANOVA showed that fasting ($P < .001$) and ethanol treatment ($P < .001$) increased soleus 7β-OH, and a significant interaction ($P = .005$) was observed. Post hoc pairwise comparisons revealed 1-day–fasting 7β-OH concentrations to be significantly higher than 2-day fasting ($P < .001$) and fed ($P < .001$) observation points. Two-day–fasting 7β-OH concentrations were significantly higher than fed ($P < .005$) concentrations, and simple effects analysis revealed a significant effect of ethanol treatment in the fed animals ($P < .001$) with higher concentrations of

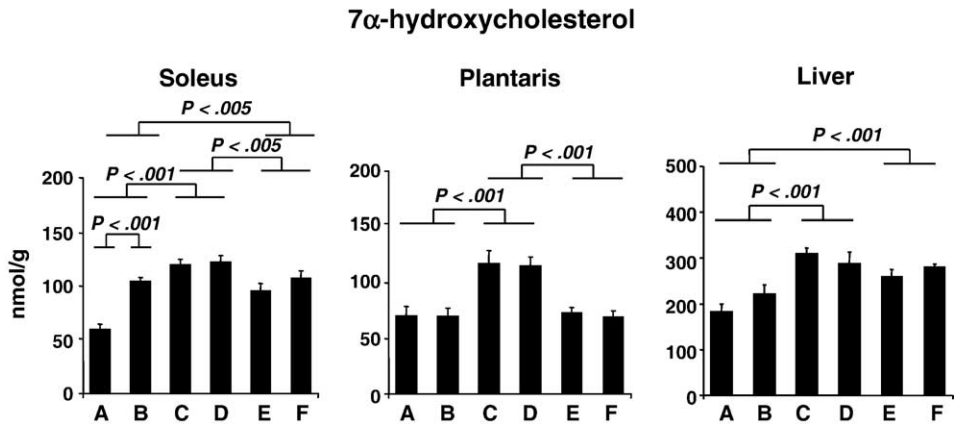

Fig. 3. Concentration of 7α-OH in muscle and liver. Oxysterols (7α-OH) were measured by HPLC-UV in muscle and liver of fed rats or those starved for 1 day (Fast 1d) or 2 days (Fast 2d). At 2.5 hours before sacrifice, rats were injected with either saline (sodium chloride given in isovolumetric amounts as ethanol-dosed rats) or ethanol (75 mmol/kg body weight IP). Data are means ± SEM nanomoles of analyte per gram of wet tissue weight (n = 5-8). A indicates fed + NaCl; B, fed + EtOH; C, Fast 1d + NaCl; D, Fast 1d + EtOH; E, Fast 2d + NaCl; F, Fast 2d + EtOH. Differences between means were assessed by ANOVA, and overall P values pertaining to the effects of ethanol, fasting, and their interactions are described in the Results section. Differences between either individual groups or pairs of groups were tested by post hoc analysis, and P values are displayed over the histograms.

7β-OH in the animals in the ethanol condition. Further simple effects analysis revealed a significant effect of ethanol treatment in the 2-day–fasting animals ($P < .05$) with higher concentrations of 7β-OH in the animals in the ethanol condition (Fig. 4).

3.2.2. Plantaris

ANOVA showed a highly significant main effect of fasting ($P < .001$). Post hoc pairwise comparisons revealed 1-day–fasting 7β-OH concentrations to be significantly higher than 2-day fasting ($P < .001$) and fed ($P < .001$) observation points (Fig. 4).

3.2.3. Liver

ANOVA showed a highly significant main effect of fasting ($P < .001$). Post hoc pairwise comparisons revealed 1-day–fasting 7β-OH concentrations to be significantly higher than at the fed ($P < .001$) observation point. Furthermore, 2-day 7β-OH concentrations were observed to be significantly higher than at the fed ($P < .001$) observation point (Fig. 4).

3.3. Effects on 7-keto

3.3.1. Soleus

As with the other 2 analytes, ANOVA showed that both fasting and ethanol treatment increased 7-keto levels ($P < .001$ in both instances). A significant interaction ($P = .05$) was observed between fasting and ethanol treatment. Post hoc analysis showed 1-day–fasting 7-keto concentrations were significantly higher than 2-day fasting ($P < .001$) and fed ($P < .001$) observation points. Simple effects

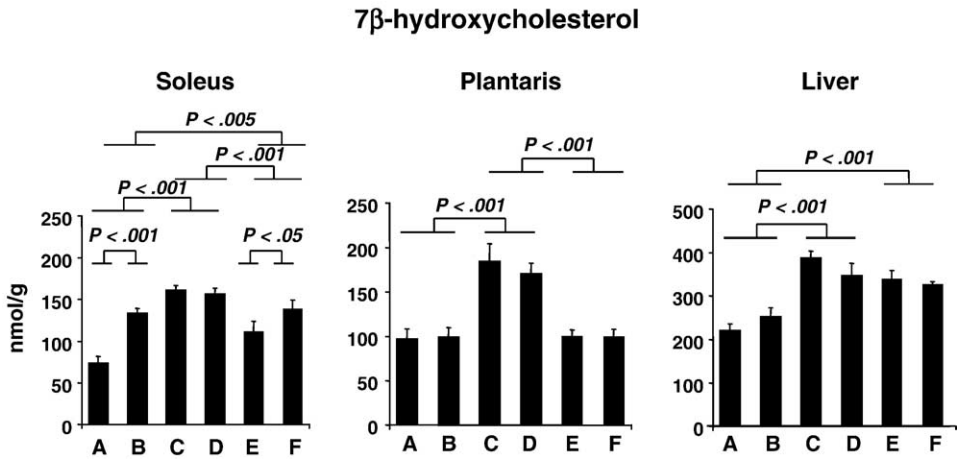

Fig. 4. Concentration of 7β-OH in muscle and liver. Oxysterols (7β-OH) were measured by HPLC-UV in muscle and liver of fed rats or those starved for 1 day (Fast 1d) or 2 days (Fast 2d). At 2.5 hours before sacrifice, rats were injected with either saline (sodium chloride given in isovolumetric amounts as ethanol-dosed rats) or ethanol (75 mmol/kg body weight IP). Data are means ± SEM nanomoles of analyte per gram of wet tissue weight (n = 5-8). A indicates fed + NaCl; B, fed + EtOH; C, Fast 1d + NaCl; D, Fast 1d + EtOH; E, Fast 2d + NaCl; F, Fast 2d + EtOH. Differences between means were assessed by ANOVA, and overall P values pertaining to the effects of ethanol, fasting, and their interactions are described in the Results section. Differences between either individual groups or pairs of groups were tested by post hoc analysis, and P values are displayed over the histograms.

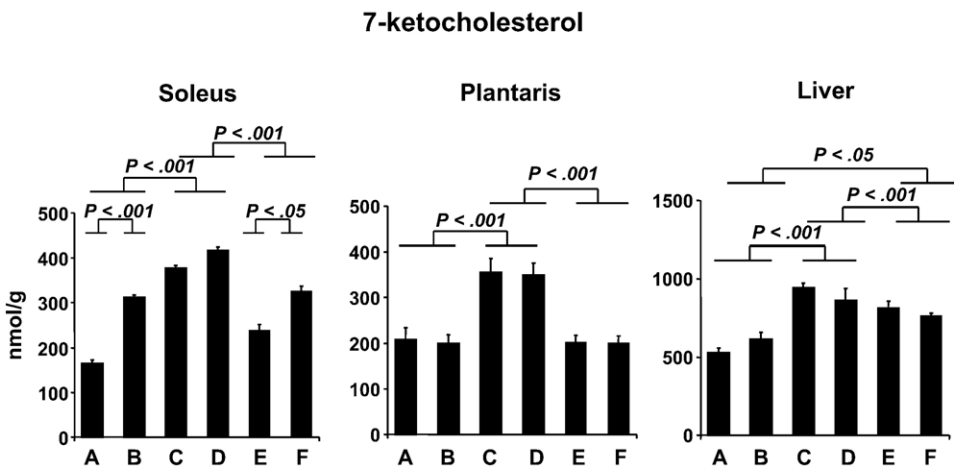

Fig. 5. Concentration of 7-keto in muscle and liver. Oxysterols (7-keto) were measured by HPLC-UV in muscle and liver of fed rats or those starved for 1 day (Fast 1d) or 2 days (Fast 2d). At 2.5 hours before sacrifice, rats were injected with either saline (sodium chloride given in isovolumetric amounts as ethanol-dosed rats) or ethanol (75 mmol/kg body weight IP). Data are means ± SEM nanomoles of analyte per gram of wet tissue weight (n = 5-8). A indicates fed + NaCl; B, fed + EtOH; C, Fast 1d + NaCl; D, Fast 1d + EtOH; E, Fast 2d + NaCl; F, Fast 2d + EtOH. Differences between means were assessed by ANOVA, and overall P values pertaining to the effects of ethanol, fasting, and their interactions are described in the Results section. Differences between either individual groups or pairs of groups were tested by post hoc analysis, and P values are displayed over the histograms.

analysis revealed a significant effect of ethanol treatment in the fed animals ($P < .001$) with higher concentrations of 7-keto in the animals in the ethanol condition. Further simple effects analysis revealed a significant effect of ethanol treatment in the 2-day–fasting animals ($P < .05$) with higher concentrations of 7-keto in the animals in the ethanol condition (Fig. 5).

3.3.2. Plantaris

ANOVA showed a highly significant main effect of fasting ($P < .001$). Post hoc pairwise comparisons revealed 1-day–fasting 7-keto concentrations to be significantly higher than 2-day–fasting ($P < .001$) and fed ($P < .001$) observation points (Fig. 5).

3.3.3. Liver

ANOVA showed a highly significant main effect of fasting ($P < .001$). Post hoc pairwise comparisons revealed 1-day–fasting 7-keto concentrations to be significantly higher than at the fed ($P < .001$) observation point and at the 2-day observation point. Furthermore, 2-day 7-keto concentrations were observed to be significantly higher than at the fed ($P < .05$) observation point (Fig. 5).

4. Discussion

We reiterate the central hypothesis that the concentrations of oxysterols in tissue increase as a consequence of food deprivation and alcohol dosage. In the following discussion, we raise issues pertaining to anorexia, food deprivation, ethanol, muscle types, and finally the implications of increased oxysterol concentrations. We measured 7α-OH, 7β-OH, and 7-keto as representative oxysterols, and they can all be assayed by the present HPLC methods. Of course, there are other oxysterols such as 19-OH, 25-OH, and 26-OH, but more sophisticated techniques such as mass spectrometry are required for their quantitative determination.

4.1. Anorexia in pathological conditions

In these studies, we sought to determine if food withdrawal elevated concentration of oxysterols in liver and muscle. These 2 tissues were selected as they are major contributors to whole-body physiology. The relevance of inducing starvation pertains to the fact that episodic reductions in food intake occurs in numerous conditions such as pulmonary disease [21], cancer cachexia [22,23], kidney dysfunction and failure [24], aging [25], HIV/AIDS [26], and a variety of gastrointestinal pathologies [27]. Because of the need to immediately fix the tissues after dissection, to inhibit enzymes and other biochemical processes that would otherwise give rise to artifactual observations on concentrations of cholesterol moieties (for a fuller discussion, see Ref. [28]), such studies are difficult to perform in a clinical setting. To resolve this, we carried out appropriate studies in laboratory animals by subjecting rats to short-term periods of fasting of 1 or 2 days and rapidly dissecting tissues. We assayed 7α-OH, 7β-OH, and 7-keto using a well-described method in which the conformational signatures of these oxysterols were recently confirmed by liquid chromatography–mass spectrometry [17]. In addition, we used a well-characterized short-term alcohol-dosing regimen, which also increases oxidative stress in muscle and liver as well as a variety of other tissue systems [16,29-33].

4.2. Effects of food deprivation

The studies showed that, in general, the tissue concentrations of oxysterols increased in fasting. For skeletal muscle, the increases were blunted or ameliorated after 2 days when compared with data from rats starved for 1 day.

In contrast, the increases in liver after 1 day of fasting were relatively sustained after 2 days. These data are not because of circulating oxysterols. This supposition is derived from the studies where we used HPLC to measure oxysterols in plasma, liver, kidney, and heart of rats. However, there was no measurable oxysterols in plasma, although they were quantifiable in the aforementioned tissues (Adachi et al, unpublished data).

Studies have been carried out on the clearance of oxysterols in rats using isotopic labeling [34]. At 5 minutes after injection of radiolabeled 7α-OH, only about 1% remains, indicating very rapid clearance kinetics, uptake, and metabolism. Similarly, 7β-OH is also rapidly cleared, that is, 8% remained 5 minutes after administration [34]. However, there is a general paucity of information on how formation, clearance, uptake, and metabolism alter under different nutritional conditions. Our results show however that fasting may influence a component of this.

As far as we are aware, this is the first report of an effect of undernutrition on tissue concentrations of oxysterols in mammalian tissues. Increased oxysterol concentrations may be because of an elevation in de novo formation as rats were totally starved. However, there is also the possibility that reduced degradation or clearance may also have been instrumental in increasing oxysterol tissue concentrations. Although the pathways for oxysterol formation are relatively well known, their routes of degradation do not appear to be have been described in detail [28,35,36].

4.3. Effects of ethanol

A number of studies have shown that a variety of free radicals are generated in the liver, and antioxidant status is impaired, as a consequence of alcohol exposure [37-39]. However, in the present studies, the liver did not show any changes in oxysterol concentration in response to a short-term ethanol load. This may reflect the higher antioxidant capacity of this tissue, or alternatively, localized effects within the liver membranes may be masked by our more global analysis of entire hepatic homogenates, which will also include intracellular components. The skeletal muscle showed increased oxysterols in response to ethanol, albeit in soleus.

4.4. Differential sensitivity of muscle types

We were somewhat surprised to see increased oxysterols in the type I soleus in response to ethanol, but there was no effect in the type II plantaris. Paradoxically, it is the type II fibers that are particularly sensitive to the deleterious effects of alcohol [16,17,40,41]. Numerous studies, including those from our group, have shown that type I fiber–predominant muscles (eg, represented by red portion of the gastrocnemius or soleus) have greater concentrations of catalase, glutathione peroxidase, superoxide dismutase (both mitochondrial and cytosolic), and α-tocopherol than type II fiber–predominant muscles (ie, the extensor digitorum longus, plantaris, or white portions of the gastrocnemius) [42-46]. This might explain why, in clinical studies in man and experiments in rats, the type II fibers are preferentially targeted by alcohol [16,47,48].

In our studies, the data show that with respect to the increased oxysterols, type I fiber–predominant muscles were more sensitive to ethanol. However, there are a number of studies that support the contention that in some selective conditions, type I fibers are more sensitive than the type II fibers in terms of their biochemical response. For example, in the long-term situation of sustained alcohol feeding for 6 weeks, type I soleus shows greater increases in oxysterols compared with the type II plantaris [17]. Furthermore, there are also other studies in which the soleus shows a greater metabolic stress response than the plantaris. For example, in experimental burn injury in the rat, increases in protein synthesis and degradation are much greater in the soleus than the plantaris [49]. Indeed, some studies have postulated that there is no relationship between fiber type and degree of biochemical injury in metabolic stress such as ischemia-reperfusion injury [50]. This is compatible with the present observations and could theoretically be explained by the fact that other major antioxidant systems such as the imidazole dipeptides (anserine and carnosine) are higher in type II muscles. They occur in millimolar concentrations [51] and confer both cytosolic buffering [52] and antioxidant properties [53-56] (in this instance, type II atrophy and loss of protein). Part of the problem in placing all these studies in the context of antioxidant or defense systems is that we do not know where in the muscle cell the free radicals are being generated in these models.

We are confident in the modeling of using anatomically distinct skeletal muscles to represent the different fiber types. In a systematic investigation, we showed virtually all aspects of the clinical situation could be reproduced in the animal model of alcohol-induced muscle disease [57].

A variety of studies have shown that in terms of oxidative stress, the liver exhibits a greater defense capacity than skeletal muscle in conditions such as sepsis [58]. However, it is important to point out that the type of oxidative stress is instrumental in determining differential responses of liver and muscle. For example, exhaustive exercise (which primarily involves skeletal muscle) and endogenous acetaldehyde generation (where the liver is the primary site of its formation and metabolism) would be expected to have contrasting effects. Overall, there is substantive evidence that the antioxidant capacity of the liver is greater than that of the muscle [59].

4.5. Implications of increased oxysterol concentrations

In considering the impact of food deprivation on oxysterols (and indeed the effects of short-term ethanol in the soleus), we must consider 2 potential implications. The first pertains to the fact that oxysterols are well-characterized agents that impart cellular damage, that is, cytotoxic agents in a number of pathways and signaling

processes. For example, both 7β-OH and 7-keto perturb cell permeability and adhesion and increase apoptosis [60-62]. Oxysterols are toxic because of their ability to form covalent bonds with biomolecules [63]. We do not know if the tissue levels reported in the present study actually represent harmful concentrations. However, we do know something about the concentrations that have been used in vitro. Studies have shown that the oxysterols are toxic in micromolar concentrations [63]. In the present study, levels of the 3 oxysterols are approximately 100 to 1000 nmol/g in the variety of conditions studied in the 3 tissues, equivalent to about 125 to 1250 μmol/L (based on the assumption that the tissues are 75% water). Conceivably, this could represent biotoxicity. However, this is rather speculative because mobilized (ie, free) oxysterols have been used in cytotoxicity studies. Presently, we do not know the extent of the free or bound oxysterols in these studies, which may represent a future avenue of research. What is important is not so much the gross tissue concentration but the localized levels at the cell interface.

We also do not know if the transient effect of fasting on muscle tissue levels is indicative of a protective clearance of the compounds.

The second implication pertains to the cellular domain being targeted. A number of studies have shown that starvation-induced membrane damage and dysfunction occurs in liver [64] and muscle [65-67]. In muscle, disturbances in the cholesterol component of membrane damage the sarcoplasmic reticulum T system, resulting in perturbation in excitation-contraction coupling [68].

In the present studies, we selected the young rat for study because we have shown that virtually all of the clinical and subclinical features of alcohol-induced muscle disease can be reproduced in this model (for further insights into this disease and models, see Refs. [16,47]). We also used short-term starvation to mimic episodic anorexia. However, rats of the age used in the present study are unable to be starved for longer than 2 days as they will not survive.

In summary, for the first time, we have shown that oxysterol concentrations increase in muscle and liver in response to food withdrawal and, in the type I soleus, in response to a short-term bolus of ethanol, an inducer of oxidative stress. Oxysterols can thus be considered as putative metabolic stress parameters and thus represent oxidative imbalance, and/or disturbances in their formation and clearance. Because of the reported cytotoxic properties of oxysterols, these data are important in understanding cellular pathology because episodic anorexia and/or oxidative or nutritional stress occurs in a variety of pathologies including sepsis, cancer cachexia, ischemia, and hormonal imbalance. However, we believe that further work is needed to determine the precise subcellular locations of these oxysterols and also to determine if their increase in ethanol exposure and starvation reflect a possible increased production per se (eg, via CYP7A1) or reduced breakdown.

Acknowledgment

This work was supported in part by a Grant-in-Aid for Scientific Research from the Japanese Society for Promotion of Sciences.

We thank the Royal Society, the Japanese Society for Promotion of Sciences, and the Daiwa Anglo-Japanese Foundation for funded travel between the UK and Japan.

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