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Growth Hormone Enhances Arachidonic Acid Metabolites in a Growth Hormone Transgenic Mouse

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Abstract In a transgenic growth hormone (GH) mouse model, highly elevated GH increases overall growth and decreases adipose depots while low or moderate circulating GH enhances adipose deposition with differential effects on body growth. Using this model, the effects of low, moderate, and high chronic GH on fatty acid composition were determined for adipose and hepatic tissue and the metabolites of 20:4n-6 (arachidonic acid) were characterized to identify metabolic targets of action of elevated GH. The products of Δ-9 desaturase in hepatic, but not adipose, tissue were reduced in response to elevated GH. Proportional to the level of circulating GH, the products of Δ-5 and Δ-6 were increased in both adipose and hepatic tissue for the omega-6 lipids (e.g., 20:4n-6), while only the hepatic tissues showed an increase for omega-3 lipids (e.g., 22:6n-3). The eicosanoids, PGE_2 and 12-HETE, were elevated with high GH but circulating thromboxane was not. Hepatic PTGS1 and 2 (COX1 and COX 2), SOD1, and FADS2 (Δ-6 desaturase) mRNAs were increased with elevated GH while FAS mRNA was reduced; SCD1 (stearoyl-coenzyme A desaturase) and SCD2 mRNA did not significantly differ. The present study showed that GH influences the net flux through various aspects of lipid metabolism and especially the desaturase metabolic processes. The combination of altered metabolism and tissue specificity suggest that the regulation of membrane composition and its effects on signaling pathways, including the production and actions of eicosanoids, can be mediated by the GH regulatory axis.

Keywords Growth hormone · Arachidonic acid · Fatty acid composition

Abbreviations

ARA	Arachidonic acid
cDNA	Complementary DNA
COX	Cyclooxygenase
DHA	Docosahexaenoic acid
ELISA	Enzyme-linked immunosorbent assay
FADS	Fatty acid desaturase
FAS	Fatty acid synthetase
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GH	Growth hormone
HETE	Hydroxyeicosatetraenoic
HPLC	High-performance liquid chromatography
MMLV-RT	Moloney murine leukemia virus-reverse transcriptase
mRNA	Messenger ribonucleic acid
oMt1a-oGH	Ovine metallothionein 1a promoter driving ovine GH transgene construct
PCR	Polymerase chain reaction
PGE2	Prostaglandin $E_{2\alpha}$
PTGS	Prostaglandin H synthase
qPCR	Quantitative real time polymerase chain reaction
SCD	Stearoyl-coenzyme A desaturase
SOD	Superoxide dismutase
SREBP	Sterol regulatory element binding protein

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STAT	Signal transducers and activators of transcription
$ZnSO_4$	Zinc sulfate

Introduction

The fatty acid composition of membrane phospholipids plays an important role in the structural, functional, and signaling properties of biological membranes and has been implicated in regulation of immunologic, physiologic, reproductive and metabolic processes and the functions of tissues from liver to adipose [1]. Arachidonic acid (C20:4n-6, ARA), the most abundant long chain polyunsaturated fatty acid in membranes, appears to be the primary biologically active polyunsaturated species mediating signaling in a variety of peripheral tissues Similarly, the n-3 polyunsaturated fatty acid docosahexaenoic acid (C22:6n-3, DHA) has a role in neural and optical signaling [2].

The negative consequences of deficiencies or excesses of polyunsaturated fatty acids correlate with ARA levels [3–5]. For example, alteration in tissue arachidonate levels can quantitatively account for many of the effects of dietary fish oils due to the omega-3 fats replacing the omega-6 fats in the membranes [3, 6–8] and metabolic deficiencies of arachidonate affect the cardiovascular and immune systems [9]. Whereas all the metabolic, physiologic and immunologic roles of ARA are not yet known, the majority of recognized effects of ARA are thought to be mediated by its conversion to biologically active signal molecules, the eicosanoids [10]. ARA oxygenation by cyclooxygenases produce thromboxane and prostaglandins, while lipoxygenases produce the hydroxyeicosatetraenoic acids (HETE) and leukotrienes (reviewed in [11, 12]). ARA can be also metabolized by cytochrome P450 monooxygenases to yield the epoxyeicosatrienoic acids. These oxygenated derivatives of ARA function to maintain a variety of physiological systems, such as ion exchange in the kidney [13], gaseous exchange in the lung, vascular smooth muscle tone [14], immune cell recruitment and function [10, 15], and activation or inhibition of endocrine glands [16]. While important in specific roles of physiological functions, elevated eicosanoids are considered to be detrimental contributing to a variety of degenerative and inflammatory disease states. As a result of this link, minimizing eicosanoid synthesis is the basis for many therapeutics aimed at controlling inflammation, thrombosis, and pain. Eicosanoids are also a target for proliferative disorders including various cancers, vascular dysfunctions, and autoimmunity.

In vivo, the limiting step for eicosanoid synthesis is the availability of ARA. Although ARA levels are regulated highly within individuals, the absolute amounts vary significantly among a population. Nonetheless, little is known about the basic mechanisms that control the absolute and relative quantities of ARA within cellular membranes. While traditionally most attention has focused on diet, recent observations that polymorphisms of the various desaturase genes are associated with altered fatty acid composition of membranes and various health outcomes has redirected attention on the mechanisms by which fatty acids are metabolized, incorporated into membranes, and converted into bioactive signaling molecules [17]. The limiting steps to membrane composition are still not clear, with both diet and genotype appearing to be important [18, 19]. The importance of hormonal signaling to this interaction has not been defined.

Elevated circulating growth hormone (GH) in a growth hormone transgenic mouse model (the oMt1a-oGH transgenic mouse) reduced essential precursors of long chain polyunsaturated fatty acids (18:2n-6 and 18:3n-3) and increased desaturation and elongation products 20:4n-6 and 22:6n-3 indicating increased activity of the Δ-5 and Δ-6 desaturation enzymes [20]. These mice with chronically elevated circulating GH, relative to wild-type control mice, exhibit about a two-fold increase of ARA content in the phosphatidyl choline phospholipid pool of hepatic membranes. The phosphatidyl ethanolamine component of membrane phospholipids, while not as great a constituent of membrane phospholipids on a percentage basis as phosphatidyl choline pools, also show a modest though significant increase in ARA abundance in response to GH [20]. Arachidonate hydrolyzed from membrane phospholipids of the phosphatidyl choline compartment by phospholipase A2 can be metabolized to eicosanoids thereby serving as local or paracrine effectors [13]. A shift toward a greater proportion of ARA in membrane compartments that are the source of liberated ARA used for subsequent metabolism should correspond to an increase in ARA metabolites. The metabolites of ARA have a wide range of physiological, immunological, neurological and reproductive functions. Therefore, a GH-specific increase in ARA could alter many biological activities, even potentiating the various negative consequences associated with inappropriately elevated eicosanoids.

Dependent upon the degree of transgene induction in the oMT1a-oGH transgenic mouse model, differential adipose phenotypes can be generated: chronically highly expressed circulating GH generates a physically larger, leaner animal [21] while minimal expression of the transgene results in mice of normal in body weight but having enlarged fat depots [22] and moderate transgene stimulation induces an intermediate level of circulating oGH corresponding to a physically larger, yet obese animal [21]. These findings suggest that circulating GH affects growth and adipose

accretion profiles differentially, dependent upon the concentration of circulating GH. Because highly elevated GH induces a change in the fatty acid composition of membranes toward a more unsaturated profile [20, 23], we wanted to determine the extent of GH action on those membranes. Therefore, we asked if long-term different levels of circulating GH, known to alter body composition and fat mass, could change lipid characteristics, and alter the products derived from the arachidonate phosphatidyl pools. Further, we assessed whether genes predicted to respond to changes in the desaturation of tissue lipids were also affected.

Materials and Methods

Animals

Mice used in this study were produced by mating wild-type C57Bl/6 × CBA female mice with males hemizygous for the oMt1a-oGH transgene. This mating scheme produces an average of 50% hemizygous transgenic progeny and 50% homozygous wild-type (i.e., non-transgenic wild-type control) animals. A single copy of the transgene acts in an autosomal dominant fashion to elevate GH expression when stimulated by adding zinc sulfate to the drinking water [24]. Mice were toe-notched for identification and genotyping [25] at 10 days of age and weaned at 21 days of age. All mice were maintained in an AAALAC approved facility in accordance with NIH animal use guidelines under conditions of constant temperature (21°C), humidity (55%), and a 14:10 h light:dark cycle. The study protocol and all procedures were reviewed and approved by the Institutional Animal Care and Use Committee at the University of California, Davis.

Experiment 1

To characterize the role of GH on adipose and hepatic lipid profiles, five to six females were randomly assigned to each of three transgene stimulus groups within a genotype (wild-type or transgenic): 25, 15, or 0 mM $ZnSO_4$ in the drinking water to yield different circulating GH concentrations. Females were chosen to corroborate the lipid alterations observed in studies of male oMt1a-oGH mice [20, 23]. Mice were housed two per cage and provided food (Formulab Chow 5008, Purina Mills, St. Louis, MO) and drinking water ad libitum. Mice were weighed weekly until 10 weeks of age at which time the mice were killed by CO_2 narcosis and gonadal fat pads, blood, and livers collected. Plasma was isolated from blood samples collected at time of animal collection and plasma circulating ovine GH concentrations (resulting from transgene expression) were assayed in duplicate using a double-antibody radioimmunoassay; ovine somatotropin standards were used (NIADDK O-GH-I-3) and a rabbit anti-ovine GH antisera (NIDDK-anti oGH-2) [26].

Lipid Analyses

To determine the effect of GH on modifying the unsaturated lipid pool, the fatty acid class composition was quantified for the phosphatidyl choline fraction of adipose and hepatic tissue as previously described [20]. Briefly tissue lipids were extracted from 200 mg tissue with H_2O/SDS/EtOH/hexanm (1:1:2:2, v/v). Phospholipids were separated by high performance thin layer chromatography using a $CHCl_3$/MeOH/acetic acid/H_2O (50:37.5:3.5:2.0, v/v) solvent system. Samples and standards (Sigma Chemical Co., St. Louis, MO) were visualized and specific lipid-containing bands collected for methylation and gas chromatography separation. The analytical methods used [27] were the most appropriate to quantify the absolute amounts of lipid species present and were designed to be highly quantitative with a cocktail of internal standards and surrogates added quantitatively to each sample. The extractions, derivatizations, and analyses were conducted on platforms to minimize quantitation problems associated with ionization efficiency. Additional hepatic tissue was flash frozen and stored at −80 °C for mRNA analyses.

RNA Analyses

The mRNA levels of genes associated with fatty acid profiles were also assessed: FADS2 (Δ-6 desaturase), fatty acid synthetase (FAS), SCD1 (stearoyl-coenzyme A desaturase 1), and SCD2 (also named Δ-9 desaturase). Several enzymes regulate the production of ARA metabolites, therefore genes expected to change in response to alterations in the desaturation status of tissue lipids were also assessed: prostaglandin H synthase (PTGS) 1 and 2 (also known as cyclooxygenase (COX-1 and 2), and superoxide dismutase 1 (SOD1). RNA was analyzed by Northern blot analysis for SCD2, FAS, SOD1, and COX-1. All reagents, unless noted, were from Sigma Chemical Company (St. Louis, MO). The RNA probes were as follows: SCD2 was as described [28], FAS was a gift from S.D. Clarke [29], Cu–Zn SOD (SOD1) was from the American Type Tissue Collection, and a 1,257 bp fragment of the murine COX-1 (PTGS1) was synthesized using 5′-TTCCTGATTCAAAAGAAGTTCTGG-3′ as the forward primer and 5′-ATGGTGGCTGTTTTGGTAGGCTGT-3′ as the reverse primer on reverse transcribed RNA isolated from murine liver using published methodology [30]. Hepatic FADS2 (Δ-6 desaturase) and SCD 1 mRNA levels were assessed by quantitative real time PCR using

published primers [31, 32], respectively) and mouse specific primers for COX-2 (PTGS2, NM_011198.3) were designed using the NCBI Primer-blast tool: forward 5′-GGCTGTTGGAATTTACGCAT-3′ and reverse 5′-CAGGGCCTTCAAAATGTCTA-3′. The internal endogenous control was GAPDH and used published primers [33]. Primers were fluorescently labeled and the mRNA was transcribed to cDNA with the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA) containing oligo d(t) and random-hexamer primers following the manufacturer's recommendations. All real-time qPCR reactions were run in 96-well plates using UDG-Supermix^{TM} (Invitrogen, Carlsbad, CA). Each reaction contained 10 μM of each primer (forward and reverse), and 5 μl diluted cDNA, in a final volume of 50 μl. The samples were amplified in an MJ Research Chromo 4TM Detector (BioRad, Hercules, CA) and fluorescence was collected during each plate read immediately following the annealing period at 60°C. All reactions were run in triplicate and average values used in quantifying the relative expression of the genes. Levels of mRNA for the genes evaluated were not different across zinc treatments in the wild-type animals ($p > 0.1$) and therefore the data were pooled and the relative quantification of the target genes was determined by the comparative Ct (ΔΔCT) method using the wild-type animal samples as the calibrator. Both the calibrator and samples from the transgenic animals were normalized to GAPDH.

Experiment 2

To explore the consequences of increased ARA levels in tissue, lungs which represent an excellent model to evaluate the production of lipoxygenase metabolites were collected from three adult male transgenic and three adult male wild-type mice maximally stimulated to express GH (25 mM $ZnSO_4$) for 7 days. Nakumura et al. [23] reported a 2.5-fold increase in liver and adipose Δ-6 desaturase activity within 7 days; therefore, this time frame was selected to evaluate the short term consequences on the products derived from the arachidonate phosphatidyl pools. Lungs were homogenized in four volumes of 0.05 M phosphate buffer (pH 7.4) and incubated for 10 min at 37°C. The lipoxygenase metabolite 12-hydroxyeicosatetraenoic acid (12-HETE) was extracted and analyzed by HPLC and diode array detection as described [34]. The effects of GH on circulating prostaglandin $E_{2\alpha}$ (PGE2) and thromboxane were determined for 15 adult male transgenic and 15 adult male wild-type mice supplemented with 25 mM $ZnSO_4$ for 3 weeks. An additional 15 adult male transgenic mice were supplemented with 0 mM $ZnSO_4$ for the same time period. The objective was to evaluate the effects of the transgenically derived GH and as such all wild-type mice received the transgene stimulus to control for any potential zinc effects on PGE2 or thromboxane. Male mice were used to avoid the confounding estrogen effects. Blood samples were collected by cardiac puncture immediately after sacrifice, plasma processed, derivatized and assayed using commercially available ELISA kits according to manufacturer's directions (Amersham, UK). Circulating GH was determined as described.

Statistical Analyses

Data were analyzed by least-squares analysis of variance procedures using PROC GLM of SAS (Version 9.1, 2004) with fixed effects of genotype, zinc stimulation level, and their interaction. Data are presented as means ± standard error of the mean. Post-hoc analysis was done using a *t* test with a Bonferroni adjustment. Significance was defined as $p < 0.05$.

Results

Experiment 1

Circulating ovine (transgene derived) GH in these transgenic mice was correlated directly with the concentration of zinc stimulus. Circulating ovine GH was 750.53 ± 43.83, 131.98 ± 40.40, and 49.22 ± 37.68 ng/ml for 25, 15, and 0 mM $ZnSO_4$, respectively. Zinc, regardless of the level (0, 15, or 25 mM), did not significantly alter body weight nor fat pad depots in the wild-type control mice used in the present study. The body weights associated with the circulating GH were 32.5 ± 0.4, 31.7 ± 0.6, and 22.2 ± 0.4 g for 25, 15, 0 mM $ZnSO_4$, respectively; wild-type mice across all zinc levels weighed 22.6 ± 0.4 g. This was reported in a previously published study of these mice [21].

Adipose Lipid Profiles

The composition of phosphatidyl choline esterified fatty acids was calculated as a mole percent for each lipid species. All three zinc treatments (0, 15, and 25 mM $ZnSO_4$) did not significant alter the adipose lipid profiles in the wild-type animal ($p > 0.1$); as such, the values for wild-type animals were pooled regardless of the zinc treatment given. The fatty acid composition of adipose lipids is presented in Table 1. In most cases, the profile for wild-type animals was generally equivalent to that for the transgenics without transgene stimulation (0 mM $ZnSO_4$) with some exceptions. In particular, the fatty acids typically associated with de novo lipogenesis, 14:1n-7 and 16:1n-7, were elevated significantly while 18:0 was reduced. These same fatty acids were also significantly

Table 1 Adipose lipid profile as mole percent for wild-type (WT) mice and transgenic (TG) mice supplemented with either 0, 15, or 25 mM $ZnSO_4$ to induce variable levels of chronic circulating GH

Fatty acid species	WT ($n = 9$)	TG + 0 mM $ZnSO_4$ ($n = 5$)	TG + 15 mM $ZnSO_4$ ($n = 4$)	TG + 25 mM $ZnSO_4$ ($n = 5$)	Model significance
14:0	2.27 ± 0.06	2.19 ± 0.09	2.22 ± 0.09	1.97 ± 0.08	$p < 0.13$
14:1n-7	0.21 ± 0.01^{ab}	0.23 ± 0.01^{bc}	0.25 ± 0.01^{c}	0.18 ± 0.01^{a}	$p < 0.003$
16:0	26.01 ± 0.46	25.93 ± 0.62	27.49 ± 0.68	25.25 ± 0.61	$p < 0.11$
16:1n-7	4.83 ± 0.22^{a}	5.69 ± 0.29^{a}	7.38 ± 0.32^{b}	4.67 ± 0.29^{a}	$p < 0.001$
18:0	5.03 ± 0.15^{bc}	4.59 ± 0.20^{ab}	4.07 ± 0.22^{a}	5.56 ± 0.22^{c}	$p < 0.001$
18:1n-9	33.4 ± 3.87	37.93 ± 5.20	35.72 ± 5.72	31.12 ± 5.07	$p < 0.836$
18:1n-7	6.80 ± 5.20	0.82 ± 10.41	2.48 ± 10.24	11.28 ± 7.18	$p < 0.841$
18:2	17.86 ± 0.22^{b}	19.14 ± 0.29^{c}	15.58 ± 0.32^{a}	16.40 ± 0.28^{a}	$p < 0.001$
18:3n-6	0.12 ± 0.00^{a}	0.13 ± 0.01^{ab}	0.14 ± 0.01^{bc}	0.15 ± 0.01^{c}	$p < 0.001$
18:3n-3	0.92 ± 0.31	1.07 ± 0.42	2.07 ± 0.46	0.70 ± 0.41	$p < 0.183$
20:0	0.11 ± 0.01^{b}	0.10 ± 0.01^{ab}	0.08 ± 0.01^{ab}	0.07 ± 0.01^{a}	$p < 0.011$
20:1	0.72 ± 0.07	0.45 ± 0.09	0.43 ± 0.10	0.42 ± 0.09	$p < 0.040$
20:2	0.18 ± 0.01^{a}	0.16 ± 0.01^{a}	0.18 ± 0.01^{a}	0.24 ± 0.01^{b}	$p < 0.001$
20:3	0.15 ± 0.00^{b}	0.13 ± 0.01^{a}	0.12 ± 0.01^{a}	0.13 ± 0.01^{a}	$p < 0.002$
20:4n-6	0.25 ± 0.01^{a}	0.32 ± 0.02^{b}	0.47 ± 0.02^{c}	0.60 ± 0.02^{d}	$p < 0.001$
20:5n-3	0.15 ± 0.01	0.18 ± 0.02	0.20 ± 0.02	0.21 ± 0.02	$p < 0.162$
22:0	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.04 ± 0.00	$p < 0.196$
22:1	0.04 ± 0.00	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	$p < 0.129$
22:4	0.05 ± 0.00^{a}	0.05 ± 0.01^{a}	0.07 ± 0.01^{a}	0.10 ± 0.01^{b}	$p < 0.001$
22:5n-3	0.20 ± 0.01	0.15 ± 0.02	0.19 ± 0.02	0.15 ± 0.02	$p < 0.118$
24:0	0.00 ± 0.00	0.00 ± 0.00	0.000 ± 0.00	0.16 ± 0.07	$p < 0.323$
22:6n-3	0.68 ± 0.06	0.68 ± 0.09	0.82 ± 0.09	0.56 ± 0.08	$p < 0.279$
24:1	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	$p < 0.621$
18:1n-9/18:0	6.65 ± 0.78	8.26 ± 1.04	8.779 ± 1.17	5.59 ± 1.26	$p < 0.133$
20:4n-6/18:2	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}	0.03 ± 0.00^{b}	0.04 ± 0.00^{b}	$p < 0.001$
22:6n-3/18:3n-3	0.74 ± 0.08	0.63 ± 0.11	0.40 ± 0.07	0.80 ± 0.13	$p < 0.887$

Zinc treatment had no effect on lipid profiles in wild-type mice ($p > 0.1$) therefore the wild-type data represent values pooled over all three zinc treatments. Values are least square means ± sem; means carrying different superscripts within a row are significantly different ($p < 0.05$)

changed in mice receiving 15 mM $ZnSO_4$. In general, substrates of the Δ-5 and Δ-6 pathways were reduced in proportion to circulating GH ($p < 0.05$). Specifically, animals with the greatest circulating GH levels had reduced 18:2 and 20:3 (~10% reduction) with elevated 20:4n-6 (~2.4-fold increase); this pattern, though not the extent, was also true for the moderate level of GH (15 mM $ZnSO_4$). In contrast, animals exposed to the lowest level of chronic GH (transgenics receiving 0 mM $ZnSO_4$), while having significantly elevated 20:4n-6 (~1.2-fold increase), also had higher levels of 18:2 (~8%). The ratio of ARA to its 18:2 precursor was significantly elevated in animals exposed to high GH levels (~ 3- to 4-fold increase for the 15 and 25 mM $ZnSO_4$, respectively). Omega-3 lipids were not affected by the altered GH nor was the Δ-9 pathway ($p > 0.10$).

Hepatic Lipid Profiles

Similar to that found for adipose, dietary provision of zinc did not significantly alter any parameter evaluated for wild-type animals ($p > 0.2$), therefore values for wild-type animals were pooled across zinc treatments. The fatty acid composition of hepatic phosphatidyl choline esterified fatty acids, calculated as a mole percent for each lipid species, is presented in Table 2. For fatty acids associated with de novo lipogenesis, there was no affect of circulating GH levels on the proportion of the fatty acids within the hepatic tissue. In contrast, for all 18 carbon fatty acids other than 18:3n-6, elevated GH reduced their percentage in the membrane and did so in proportion to the level of transgene expression. A concomitant increase in 20:4n-6 (~1.8-fold increase) was seen also correlating with the level of GH

Table 2 Hepatic lipid profile as mole percent for wild-type (WT) mice and transgenic (TG) mice supplemented with either 0, 15, or 25 mM $ZnSO_4$ to induce variable levels of chronic circulating GH

Fatty acid species	WT ($n = 8$)	TG + 0 mM $ZnSO_4$ ($n = 4$)	TG + 15 mM $ZnSO_4$ ($n = 5$)	TG + 25 mM $ZnSO_4$ ($n = 4$)	Model significance
14:0	0.58 ± 0.05	0.51 ± 0.08	0.51 ± 0.07	0.32 ± 0.08	$p < 0.086$
14:1n-7	0.22 ± 0.01	0.01 ± 0.01	0.04 ± 0.01	0.01 ± 0.01	$p < 0.242$
16:0	23.68 ± 2.06	24.24 ± 3.00	25.36 ± 2.63	23.15 ± 2.93	$p < 0.881$
16:1n-7	4.27 ± 1.34	2.08 ± 1.96	2.44 ± 1.71	4.52 ± 1.91	$p < 0.665$
18:0	10.75 ± 0.63^{a}	14.25 ± 0.92^{b}	13.90 ± 0.81^{b}	14.84 ± 0.90^{b}	$p < 0.006$
18:1n-9	22.91 ± 1.16^{b}	17.33 ± 1.70^{a}	18.83 ± 1.48^{ab}	17.36 ± 1.66^{ab}	$p < 0.017$
18:1n-7	2.96 ± 0.24	1.90 ± 0.36	2.20 ± 0.31	1.87 ± 0.40	$p < 0.044$
18:2	14.44 ± 0.73^{b}	14.46 ± 1.07^{ab}	11.43 ± 0.93^{ab}	9.96 ± 1.04^{a}	$p < 0.007$
18:3n-6	0.19 ± 0.01^{a}	0.27 ± 0.02^{b}	0.26 ± 0.02^{b}	0.27 ± 0.02^{b}	$p < 0.007$
18:3n-3	0.33 ± 0.03^{b}	0.34 ± 0.04^{b}	0.29 ± 0.03^{ab}	0.18 ± 0.04^{a}	$p < 0.014$
20:0	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	$p < 0.913$
20:1	0.32 ± 0.02^{b}	0.18 ± 0.03^{a}	0.24 ± 0.02^{ab}	0.25 ± 0.03^{ab}	$p < 0.003$
20:2	0.42 ± 0.02	0.32 ± 0.03	0.37 ± 0.03	0.40 ± 0.03	$p < 0.107$
20:3	1.16 ± 0.04^{c}	0.92 ± 0.06^{b}	0.69 ± 0.05^{ab}	0.55 ± 0.06^{a}	$p < 0.001$
20:4n-6	7.00 ± 0.66^{a}	9.07 ± 0.96^{ab}	10.65 ± 0.84^{bc}	12.84 ± 0.94^{c}	$p < 0.001$
20:5n-3	0.95 ± 0.08^{b}	1.26 ± 0.11^{b}	0.94 ± 0.10^{b}	0.42 ± 0.11^{a}	$p < 0.001$
22:0	0.17 ± 0.01	0.19 ± 0.02	0.17 ± 0.02	0.20 ± 0.02	$p < 0.762$
22:1	0.01 ± 0.00	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	$p < 0.232$
22:4n-6	0.16 ± 0.02^{a}	0.18 ± 0.03^{a}	0.22 ± 0.02^{a}	0.34 ± 0.03^{b}	$p < 0.001$
22:5n-3	0.48 ± 0.05	0.64 ± 0.08	0.47 ± 0.07	0.35 ± 0.08	$p < 0.139$
24:0	0.09 ± 0.02	0.12 ± 0.03	0.14 ± 0.03	0.17 ± 0.03	$p < 0.320$
22:6n-3	8.64 ± 0.54^{a}	11.35 ± 0.78^{ab}	10.42 ± 0.68^{ab}	11.53 ± 0.76^{b}	$p < 0.025$
24:1	0.31 ± 0.03	0.33 ± 0.05	0.33 ± 0.04	0.39 ± 0.05	$p < 0.494$
18:1n-9/18:0	2.13 ± 0.16^{b}	1.22 ± 0.24^{a}	1.35 ± 0.20^{ab}	1.17 ± 0.23^{a}	$p < 0.006$
20:4n-6/18:2	0.48 ± 0.08^{a}	0.63 ± 0.11^{ab}	0.93 ± 0.10^{bc}	1.29 ± 0.11^{c}	$p < 0.001$
22:6n-3/18:3n-3	26.25 ± 5.06^{a}	33.24 ± 7.04^{a}	35.53 ± 5.34^{ab}	64.77 ± 6.82^{b}	$p < 0.002$

Zinc treatment had no effect on lipid profiles in wild-type mice ($p < 0.1$) therefore the wild-type data represent values pooled over all three zinc treatments. Values are least square means ± sem; means carrying different superscripts within a row are significantly different ($p < 0.05$)

expressed from the transgene. Elevated GH reduced the 18:1/18:0 ratio by nearly 50% and the combined effects of GH raising ARA (20:4n-6) and decreasing alpha linoleic (18:2) resulted in a significantly elevated 20:4/18:2 (ARA: linoleic acid) ratio (~2.5-fold increase, $p < 0.001$). A similar result was detected for the 22:6/18:3n-3 (DHA: alpha linolenic acid) ratio ($p < 0.002$). The elevated ratios were proportional to the degree of elevated GH in circulation: the 25 mM $ZnSO_4$ treated transgenic animals had the greatest ratios followed by the 15 mM $ZnSO_4$ treated transgenics which were in turn greater than the 0 mM $ZnSO_4$; with the last group's ratios statistically equivalent to those of the wild-type controls.

Hepatic RNA

Wild-type control animals given 0, 15, and 25 mM $ZnSO_4$ supplementation did not exhibit significant differences in mRNA levels for any of the genes evaluated ($p > 0.1$) due to zinc treatment and were therefore pooled for analyses. Stearyl-CoA desaturase 1 hepatic mRNA levels were unaffected by alterations in circulating GH ($p > 0.5$) and SCD 2 levels were not significantly reduced in proportion to the level of circulating GH (Fig. 1; $p < 0.1$). Animals experiencing the highest levels of circulating GH had significantly reduced FAS mRNA compared to wild-type control animals ($p < 0.01$). The animals with low, but chronic GH had intermediate FAS mRNA values that were not significantly different than the wild-type animals that had the highest FAS mRNA levels. In contrast, SOD1 mRNA was elevated in animals expressing the highest levels of GH while animals with the low and moderately elevated GH levels had SOD1 levels equivalent to wild-type expression ($p < 0.001$). Levels of COX-1 mRNA were significantly elevated for the animals with moderate to highly elevated GH (transgenics supplemented with 15

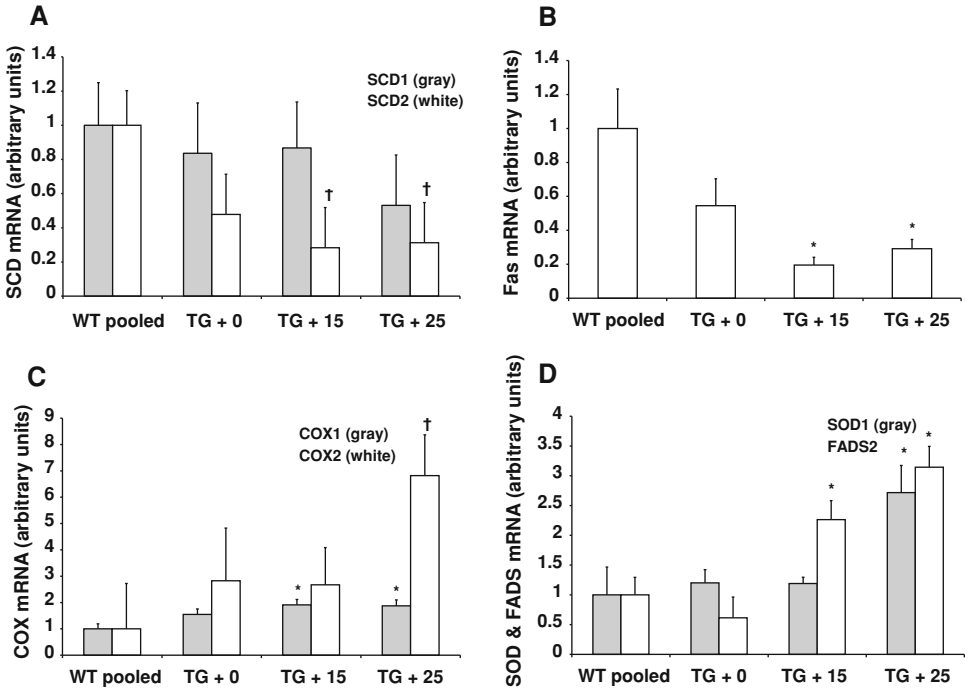

Fig. 1 Hepatic mRNA levels in oMT1a-oGH transgenic (TG) mice exposed to 0, 15, and 25 mM $ZnSO_4$ transgene stimulus used to vary the level of circulating GH as compared to wild-type control (WT) mice, pooled across all zinc treatment levels. Values are expressed as means + sem, $n = 5$–6 mice per group, and means with an *asterisk* differ from control mice $p < 0.05$; means denoted with a† differ from control mice $p < 0.1$. **a** Stearyl-CoA desaturase (SCD) 1 (*gray bars*) and 2 (*white bars*), **b** Fatty acid synthase (FAS), **c** Cyclooxygenase (COX) 1 (*gray bars*) and 2 (*white bars*), **d** Superoxide dismutase 1 (SOD1, *gray bars*) and FADS2 (*white bars*)

and 25 mM $ZnSO_4$, respectively) when compared to the wild-type animals ($p < 0.01$). Transgenic mice not given any zinc stimulus therefore having low chronic GH in circulation, had intermediate levels of COX-1 mRNA (Fig. 1). A similar trend was seen for the COX-2 mRNA levels ($p < 0.1$) with the highest mRNA levels showing a nearly a seven-fold increase, detected in the animals experiencing the greatest circulating GH. The mRNA levels of FADS2 paralleled the changes in hepatic lipid membranes: the highest GH exposure resulted in the greatest level of FADS2 mRNA ($p < 0.001$).

Experiment 2

Arachidonic Acid Metabolites

Because the transgene had not been stimulated during the growth phase of the adult males used in this experiment, body weights did not significantly differ: 42.5 ± 1.7 and 42.4 ± 0.9 g for the transgenic and wild-type mice, respectively. Upon activation of the transgene, circulating ovine GH levels in the adult transgenics were 332.0 ± 23.8 ng/ml. The arachidonic metabolite 12-HETE was significantly elevated in mice after 1 week of exposure to elevated GH ($p < 0.05$). Transgenic mice supplemented with 25 mM $ZnSO_4$ had over twice the quantity of 12-HETE in their lungs than wild-type mice also supplemented with 25 mM $ZnSO_4$ (Fig. 2). Similarly, plasma PGE2 was significantly elevated in response to high circulating GH. In contrast, the unstimulated transgenic mice

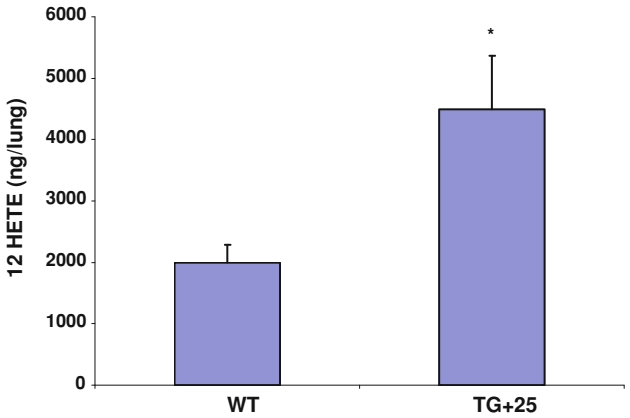

Fig. 2 Production of the 12 lipoxygenase product 12 HETE in lungs of wild-type control (WT) and oMT1a-oGH transgenic (TG) mice exposed to the transgene stimulus (25 mM zinc). Data are expressed as means + sem (ng/lung) and are significantly different ($n = 3$ per group)

with low chronic GH (0 mM $ZnSO_4$) did not exhibit any changes in PGE2 relative to the wild-type mice given 25 mM $ZnSO_4$ (Table 3). Circulating thromboxane was not significantly affected by elevated GH (Table 3).

Discussion

The effects of elevated circulating GH on the regulation of lipid profiles, specifically metabolites of the long chain saturated and polyunsaturated fatty acid desaturases, were

Table 3 Plasma arachidonic acid metabolites as a consequence of circulating growth hormone

Treatment	PGE2 (pg/ml)	Thromboxane (ng/ml)
WT + 25 mM $ZnSO_4$	13.54 ± 11.19[a] ($n = 8$)	224.8 ± 97.6 ($n = 8$)
TG + 0 mM $ZnSO_4$	13.23 ± 11.19[a] ($n = 8$)	385.3 ± 83.3 ($n = 11$)
TG + 25 mM $ZnSO_4$	49.84 ± 10.50[b] ($n = 9$)	237.1 ± 71.3 ($n = 15$)

Values are least square means ± sem; means carrying different superscripts within a column are significantly different ($p < 0.05$)

studied. In this GH transgenic model, mice exposed to moderate but chronically increased circulating GH become obese whereas highly elevated circulating GH reduces adipose stores [21] and alters hepatic membrane lipid profiles in a rapid and sustained manner [20, 23]. Although elevated GH is frequently associated with reduced adipose mass, in this model development of obesity in the presence of moderately elevated GH is thought to be mediated by greater expression of GH receptor binding protein [21] or through increased leptin and insulin secretion as seen in the moderately obese GHRH transgenic mouse that also has elevated GH levels [35].

In hepatic tissues, the activity of the Δ-9 desaturase as indicated by the lipid products was decreased in response to high circulating GH whereas the Δ-5 and Δ-6 enzymatic activities appeared to be increased in both adipose and hepatic tissues as evidenced by a greater percentage of ARA. Elevated DHA in response to GH was only detected in hepatic but not adipose tissue indicating that GH exerts tissue specificity in the processing of fatty acids. These findings corroborate previous studies evaluating hepatic lipid profiles [20, 23] although there were some discrepancies likely due to duration of GH exposure and downstream effects of the elevated GH. Specifically, short term elevated GH significantly depressed DHA and ARA in adipose, whereas the present study with long-term GH exposure did not significantly depress DHA and elevated ARA. Additionally, the data demonstrate that the GH induced change to lipid profiles occurs in both males and females. The latter is important in that women have lower Δ-6 enzymatic activities than men [36]; the finding that both male and female mice responded similarly with enhanced activity to GH suggests that GH may be a key endogenous regulator of the net flux through the desaturase pathway. The higher the circulating GH, the greater the Δ-5 and Δ-6 desaturase activity as evidenced by changes in the lipid profile and greater FADS expression. However, in this mouse model high GH is associated with elevated insulin; insulin levels decline following GH withdrawal [26] indicating that the transgenically derived GH impairs insulin signaling causing a mild diabetic state. Insulin is known to induce the expression of the Δ-5, Δ-6, and Δ-9 desaturases in rats [37] suggesting that long-term GH action observed in the present study may be mediated through elevated insulin.

An increased proportion of ARA in tissues would be expected to elevate ARA metabolites and that was the case for circulating PGE2 and lung produced 12-HETE. Similarly, the constitutively active COX1 mRNA as well as COX2 mRNA were increased in response to elevated circulating GH suggesting coordinated regulation of this pathway. In contrast, the most pro-thrombotic eicosanoid, thromboxane was unchanged despite the dramatic increase in the level of ARA, its precursor. Thromboxane has been shown to be associated directly with membrane arachidonate; specifically thromboxane is lower in animals fed alpha linolenic acid (18:3n-3) which correlates to higher levels of 18:3n-3 in the liver membranes [39] that replace 18:2 lowering overall arachidonate, a precursor of thromboxane [38]. Levels of circulating thromboxane in the current study did not significantly change with exposure to GH even though arachidonate was increased appreciably. The GH induced increase of ARA may not have been within the specific precursor pools for the thromboxane production.

Elevated highly unsaturated fatty acid species within membranes could serve as targets for reactive oxygen species and could increase the opportunities for oxidative modification and damage. As tissue membranes became more unsaturated in response to high GH, SOD1 mRNA was significantly elevated. Generally SOD1 is constitutively expressed although ARA can upregulate SOD1 gene transcription in vitro (39). High circulating GH with its shift toward unsaturated fatty acid species increased expression of SOD1 indicating that only supraphysiological arachidonic levels pose an oxidative risk and increase SOD1 expression in vivo.

Fatty acid synthetase, a key regulator of de novo long chain fatty acid biosynthesis, promotes adipose storage; inhibition of FAS in vivo results in weight loss [40]. FAS activity is regulated at the level of gene expression rather than enzymatic activation and FAS can be transcriptionally regulated by STAT5A, a GH signal transducer [41]. GH activation of STAT5 plays a defined role in adipogenesis [42] and by activating STAT5, GH can block the transcriptional activation of FAS in adipocytes [41] thereby reducing lipid accrual. GH also reduces glucose uptake thereby further reducing de novo long chain fatty acid biosynthesis and the substrates available for desaturation.

Hogan and Stephens [41] showed that GH inhibition of FAS is rapidly reversible, consistent with the observation that chronic exposure to GH produces a lean phenotype but withdrawal of GH results in the rapid accumulation of

adipose [25]. FAS mRNA was reduced in the presence of all levels of GH, even those known to promote adipogenesis and adipose accrual in these animals [21]. The increase in adipose deposition observed in these transgenic animals upon withdrawal of high GH or in response to moderate levels of GH occurs regardless of feed restriction [24] indicating that GH overrides the expected composition of gain observed for normal rodents [42] and the expectation of reduced adipose deposition associated with lowered FAS expression. Therefore, FAS expression alone is insufficient to account for adipose deposition in animals that become obese while experiencing moderately high GH levels. Although alterations in mRNA levels may not necessarily imply changes in protein activity, clearly this is an area that requires further exploration. Metabolic end-products of FAS, in particular 16:1n-7 have recently been identified as participating in tissue to tissue communication and the overall regulation of systemic energy [27]. In the present GH manipulated animals 16:1n-7 was elevated in the adipose of overweight animals and lowered in GH stimulated mice implying that an additional level of regulation by GH may be through this form of metabolic end product, i.e. lipokine signaling.

Exposure to GH did not significantly decrease hepatic SCD2 mRNA. In a study where only SCD1 mRNA was evaluated, lactating cows given recombinant bovine GH [43] had reduced SCD1 mRNA in adipose but not mammary tissue implying tissue specificity in GH modulation of SCD expression. SCD1 null mice have lowered body adiposity, depressed hepatic expression of fatty acid synthesis genes, and increased expression of fatty acid oxidation genes [44]. In response to elevated GH in the present study, SCD expression was reduced although not significantly so. FAS and fatty acid oxidation gene expression followed an expression pattern similar to that seen for the SCD1 null mouse indicating a potential mechanism of action for GH.

The interplay between circulating GH and adipose cannot be accounted for by changes in membrane lipids and their potential signaling metabolites. It is curious that GH stimulated the desaturase pathways in membrane lipids in a tissue specific manner between adipose and hepatic membranes with particular reference to the omega-3 lipids. Similarly, GH altered ARA metabolites differentially with PGE2 and 12-HETE elevated but with no effect on thromboxane. This mouse model, especially by targeting adipose tissue, may be useful in elucidating the mechanism behind the differential tissue and eicosanoid response. Particularly as the finding that the desaturase process responds to different GH levels is intriguing and suggests the possibility of modifying the pathway to minimize the negative aspects of inappropriately elevated eicosanoids.

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