Amorfrutins Are Dietary SPPARγMs with Potent Binding Affinity. To identify new dietary molecules that could act as potent antidiabetic SPPARγMs, we screened a structurally diverse natural products library consisting of approximately 8,000 pure compounds derived from edible biomaterials, using mass spectrometry detection (SI Appendix, Fig. S1A). The screen revealed 90 potential PPARγ ligands (SI Appendix, Fig. S1B), which were characterized in additional assays to confirm PPARγ binding and activation. We identified the amorfrutins, a family of isoprenoid-substituted benzoic acid derivatives without any stereocentres, as structurally new PPARγ agonists with high binding affinity (Fig. 1A). The amorfrutins were isolated from the edible roots of licorice, Glycyrrhiza foetida, which are used in traditional medicine and are widely available. We also isolated amorfrutins from fruits of another legume, Amorpha fruticosa, an ingredient of some condiments.

The PPARγ binding affinity constants of amorfrutins 1–4 ranged from 236 to 354 nM (Fig. 1B, Table 1), indicating that these compounds bind about twice as strongly to PPARγ as the synthetic drug pioglitazone (Actos, Kᵢ = 584 nM). The amorfrutins showed weaker binding to other PPAR subtypes with a selectivity factor of PPARs may promote specific gene expression profiles that result in more favorable outcomes. The use of selective PPARγ modulators (SPPARγMs) (9–11) as well as inhibition of phosphorylation of serine 273 of PPARγ by small molecules are two recently proposed approaches for improving insulin sensitivity while minimizing aforementioned side effects (12, 13).

A large proportion of drugs are based on natural products or their synthetic analogues (14), and purified natural products or extracts derived from edible biomaterials have recently become a major focus of nutrition research aiming to develop functional food and nutraceuticals with demonstrable health benefits (15).

Results and Discussion

Amorfrutins are potent antidiabetic dietary natural products


Ober the last few decades metabolic diseases such as type 2 diabetes have evolved into a global epidemic (1). Exercise and dietary regimes can counteract the development of obesity and type 2 diabetes, but complementation of such strategies with safe preventive drugs or tailored food supplements may be needed to combat the epidemic of insulin resistance, a hallmark of metabolic disease (2). The nuclear receptor PPARγ (peroxisome proliferator-activated receptor gamma) plays a central role in lipid and glucose metabolism; however, current PPARγ-targeting drugs are characterized by undesirable side effects. Natural products from edible biomaterials provide a structurally diverse resource to alleviate complex disorders via tailored nutritional intervention. We identified a family of natural products, the amorfrutins, from edible parts of two legumes, Glycyrrhiza foetida and Amorpha fruticosa, as structurally new and powerful antidiabetics with unprecedented effects for a dietary molecule. Amorfrutins bind to and activate PPARγ, which results in selective gene expression and physiological profiles markedly different from activation by current synthetic PPARγ drugs. In diet-induced obese and db/db mice, amorfutrin treatment strongly improves insulin resistance and other metabolic and inflammatory parameters without concomitant increase of fat storage or other unwanted side effects such as hepatotoxicity. These results show that selective PPARγ-activation by diet-derived ligands may constitute a promising approach to combat metabolic disease.


Conflict of interest statement: The authors declare no conflict of interest, with the exception of K. S. and L. M.-K. from Analyticon Discovery, a company that sells natural products.

This article is a PNAS Direct Submission. Data deposition: The X-ray crystallography data have been deposited in the Protein Data Bank, www.pdb.org (PDB ID code 2yfe). The microarray data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE28384).

*To whom correspondence should be addressed. E-mail: sauer@molgen.mpg.de.

This article contains supporting information online at www.pnas.orglookup/suppl/doi:10.1073/pnas.1116971109/-/DCSupplemental.

www.pnas.org/cgi/doi/10.1073/pnas.1116971109

PNAS Early Edition | 1 of 6
for PPARγ of approximately 20 to 200 (SI Appendix, Fig. S2 A and B, Table 1). For example, amorfrutin 1 has binding affinities of 236 nM for PPARγ, which are more than 100-fold higher than for PPARα and PPARβ/δ (each have a binding constant of 27 μM). However, the amorfrutins also exhibited low-micromolar activity on PPARα (which is mainly expressed in the liver) and on ubiquitously expressed PPARβ/δ, suggesting that these compounds can potentially contribute to treatment of diabetes-associated disorders such as dyslipidemia and hypercholesterolemia (16).

In contrast to the full PPARγ agonist rosiglitazone, amorfrutins induced only partial recruitment of several transcriptional cofactors including CBP, PGC1α, TRAP220/DRIP, and PRIP/ RAP250. Strikingly, amorfrutin 1 abolished recruitment of the corepressor NCoR showing IC50 values similar to those of rosiglitazone (51 nM for amorfrutin 1 vs. 64 nM for rosiglitazone, SI Appendix, Fig. S2 C–G, Table 1). As reported recently, NCoR deletion results in PPARγ activation and increased insulin sensitivity (17). We confirmed partial PPARγ activation by amorfrutins using a reporter gene assay and detected activation of 15 to 39% relative to full PPARγ activation (Fig. 1C, Table 1). Using cellular reporter gene or coactivator recruitment assays, we also tested for potential interaction with other nuclear receptors involved in adipocyte differentiation, metabolism, or xenobiotic sensing such as the estrogen receptors alpha and beta, the liver X receptor but did not detect any activation (SI Appendix, Fig. S3 A–E).

Crystal Structure of PPARγ-Binding Amorfrutin 1. To gain further insight in the interaction of amorfrutins with PPARγ, we examined the structure of the complex of the PPARγ-ligand binding domain (LBD) and amorfrutin 1 by X-ray crystallography (2.0 Å resolution). In the resulting dimeric structure, polypeptide chain ‘B’ of PPARγ-LBD was distorted due to crystal contacts, consistent with previously published PPARγ structures (18–20). The other chain ‘A’ contained an amorfrutin 1 molecule bound between helix H3 and the β-sheet (Fig. 2A). The PPARγ-LBD recognized natural amorfrutin 1 in a similar way as the synthetic partial agonists nTZDpa, MRL-24, and BVT13. All of these ligands stabilized helix H3 and the β-sheet and were linked to Ser342 and Arg288 of the LBD via hydrogen bonds and salt bridges (20) (Fig. 2B, SI Appendix, Fig. S4A). Disruption of these interactions by methylating the carboxyl group in amorfrutin weakened the binding to PPARγ by a factor of 40 (SI Appendix, Fig. S4B).

The structure also revealed that the ortho-phenyl and meta-isoprenyl residues of amorfrutin 1 have extensive van der Waals contacts with the LBD.

Amorfrutins Selectively Modulate PPARγ Gene Expression Networks in Adipocytes. Consistent with partial activation of PPARγ in vitro and the observation of amorfrutin-LBD binding in the X-ray structure, we confirmed activation of expression of known PPARγ target genes by the amorfrutins. Classical PPARγ target genes such as Fabp4, Slc2a4, and Nr1h3 were upregulated in differentiated adipocytes but to a much lower degree compared to rosiglitazone (SI Appendix, Fig. S5A). Knockdown of PPARγ reduced significantly or abolished amorfrutin-induced gene expression, suggesting specific activation of PPARγ-dependent gene expression networks by these natural products (SI Appendix, Fig. S5B).

Upregulation of PPARγ target genes by amorfrutins was in general weaker than for the full agonist rosiglitazone, in concomitant with markedly less pronounced adipocyte differentiation. We further compared gene expression profiles of human primary adipocytes treated with amorfrutins, the full PPARγ agonists rosiglitazone and pioglitazone, and the selective PPARγ modulators nTZDpa (21) and telmisartan (22). Gene Ontology and Gene Set Enrichment Analysis (GSEA) revealed molecular networks of PPARγ modulation by amorfrutins. The most enriched pathway for amorfrutin 1 and 2 was the PPAR signaling pathway. Gene distance matrix comparison (Fig. 2C), hierarchical clustering (SI Appendix, Fig. S5C), and principal component analyses (SI Appendix, Fig. S5 D and E) strongly support classification of the amorfrutins as natural PPARγMs, showing characteristically different expression patterns compared to known synthetic PPARγ agonists. Notably, gene expression profiles of amorfrutins 1 and 2 were partially distinct, indicating that small changes in ligand structure may contribute to fine tuning of transcriptional regulation (Fig. 2D). Cholesterol biosynthesis, fatty acid elongation, and fatty acid oxidation genes were efficiently upregulated by amorfrutin treatment. In contrast, inflammation pathways were downregulated (Fig. 2E, SI Appendix, Fig. S5 F and G). As for many approved drugs and natural products (23, 24), we can of course not completely rule out the possibility of off-target effects of the amorfrutins; for example the inhibition of NF-κB pathways in some cells (25). Nevertheless, our in vitro results and detailed analyses of gene expression data, including application of the Connectivity Map approach (26) for drug discovery, strongly suggested that the amorfrutins act mainly as insulin sensitizers (SI Appendix, Table S2).

Amorfrutins Act as Antidiabetics in Mouse Models for Type 2 Diabetes. Next, we evaluated the in vivo effects of amorfrutin 1 on insulin resistance in high-fat diet-induced obesity (DIO) C57BL/6 mice.

Table 1. Affinity constants (Ki), effective concentrations (EC50) and efficacy of investigated compounds binding to various PPAR subtypes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>PPARα</th>
<th>PPARβ/δ</th>
<th>PPARγ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ki [μM]</td>
<td>Ki [μM]</td>
<td>EC50 [μM]</td>
</tr>
<tr>
<td>Amorfrutin 1</td>
<td>27</td>
<td>27</td>
<td>0.236</td>
</tr>
<tr>
<td>Amorfrutin 2</td>
<td>25</td>
<td>17</td>
<td>0.287</td>
</tr>
<tr>
<td>Amorfrutin 3</td>
<td>115</td>
<td>68</td>
<td>0.352</td>
</tr>
<tr>
<td>Amorfrutin 4</td>
<td>8</td>
<td>6</td>
<td>0.278</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.007</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.584</td>
</tr>
<tr>
<td>nTZDpa</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.029</td>
</tr>
<tr>
<td>GW0742</td>
<td>n.d.</td>
<td>0.0004</td>
<td>n.d.</td>
</tr>
<tr>
<td>GW7647</td>
<td>0.001</td>
<td>0.180</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

Ki values were obtained by using a competitive TR-FRET assay, EC50 and efficacy values were determined from a reporter gene assay.

Efficacy is the maximum activation relative to the rosiglitazone-induced activation of PPARs, n.d., not determined.
For this purpose, we developed a chemical synthesis that provided multigram quantities of amorfrutin 1 of greater than 99% purity (see SI Appendix, Methods). A panel of ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) studies did not reveal any adverse effects of amorfrutin 1 application (SI Appendix, Fig. S64, Table S3). Furthermore, using an in vitro micronucleus assay, we observed no genotoxicity of amorfrutin 1 at physiologically relevant doses (SI Appendix, Fig. S6B). After feeding a high-fat diet (HFD) for 12 w, the DIO mice were treated for 23 d with 100 mg/kg/d amorfrutin 1, a dosage for which we anticipated antidiabetic and nontoxic effects based on the affinity to PPARγ and the ADMET properties observed. In the mouse studies, liver toxicity indicating plasma alanine transaminase (ALT) assays showed reduced ALT levels in mice treated with amorfrutin 1 compared to mice treated with vehicle control or rosiglitazone (SI Appendix, Fig. S6C). Similarly, whole-genome expression analysis of mice livers suggested no toxic effects after amorfrutin treatment (SI Appendix, Fig. S6D). Amorfrutin 1 and rosiglitazone both showed equal reduction of insulin resistance in DIO mice as assessed by homeostatic modelling (Fig. S4). Amorfrutin 1 considerably enhanced glucose tolerance [19% decrease in glucose area under the curve (AUC), 42% decrease in insulin AUC vs. vehicle] and insulin sensitivity (14% increase in glucose AUC, vs. vehicle) during oral glucose tolerance tests (OGTT, Fig. 3B) and intraperitoneal insulin sensitivity tests (IPIST, Fig. 3C). Moreover, amorfrutin 1 strongly decreased plasma triglycerides, free fatty acids, insulin, and glucose comparable to rosiglitazone (Fig. 3D, SI Appendix, Fig. S7A).

Both rosiglitazone and amorfrutin 1 increased food intake (SI Appendix, Fig. S7B) as previously described for PPARγ ligands (27). But in contrast to rosiglitazone, amorfrutin 1 treatment over three weeks reduced significantly body weight gain in DIO mice by approximately 10% compared to DIO mice treated with vehicle control (Fig. 3E). Such a surprising effect has also been reported for the pan-PPAR agonist bezafibrate (28), while for many synthetic SPPARγMs reduced food consumption and concomitantly decreased weight gain have been observed (29). The reduced weight gain in our DIO mice was associated with slightly elevated plasma concentration of thyroxine (T4), a marker for increased energy expenditure (SI Appendix, Fig. S7C). Because the complex effects of PPARγ agonism on various endocrine systems and downstream physiological changes (e.g., change in thermogenesis, fatty acid oxidation, or activity) are not fully understood, it is difficult to probe all potential mechanisms by which the amorfrutins may affect weight regulation. For example, recent studies suggest that complex interaction of brain PPARγ-signaling with peripheral organs may contribute to the physiological regulation of energy balance (30, 31). Presumably, the amorfrutins as partial agonists may act on neuronal PPARγ by antagonising diet-derived endogenous agonists such as fatty acids, thereby leading to relative weight loss. Notably, in our study an increase in food intake became apparent not until day 10 of the treatment with amorfrutin, whereas beneficial reduction of weight gain already started during the first days. Orexigenic effects may therefore be secondary to weight gain reduction.

We also investigated the antidiabetic effects of amorfrutin 1 in leptin receptor-deficient db/db mice, a genetic model of severe diabetes. In this model, rosiglitazone strongly increased body weight by approximately 30% within 3 w, whereas amorfrutin 1 treatment had no significant effects on mouse body weight (SI Appendix, Fig. S7D). Strikingly, amorfrutin 1 reduced plasma insulin concentrations more strongly than rosiglitazone (36% vs.
Amorfrutins Inhibit HFD-Induced PPARγ Ser273 Phosphorylation in Mouse Adipocytes. Phosphorylation by protein kinase Cdk5 at serine 273 of PPARγ in adipocytes leads to dysregulation of a large number of genes whose expression is altered in obesity (12). Inhibition of the Ser273-phosphorylation was thus proposed as a new strategy to increase insulin sensitivity specifically, without activating the full range of PPARγ targets, and thereby avoiding known side effects such as weight gain. Phosphorylation of PPARγ in visceral white adipose tissue of DIO mice was blocked by amorfrutin 1 (Fig. 4A). This effect was significantly correlated with improved insulin sensitivity (Fig. 4B). As shown above, compared to rosiglitazone the amorfrutins do not induce expression of large gene sets (Fig. 2D), leading for example to reduced expression of genes for fat storage such as Fabp4. We further observed in vivo that amorfrutin 1 more efficiently than rosiglitazone counterregulated a set of 17 genes (Fig. 4C) that had recently been reported (12) to be altered by HFD-induced activation of the kinase Cdk5 in white adipose tissue, which is consistent with decreased PPARγ-Ser273 phosphorylation. Thus, the amorfrutins were more efficient than rosiglitazone in reversing the gene expression changes induced by high-fat diet. The striking inhibition of NCoR recruitment by amorfrutin 1, as revealed by cofactor recruitment analysis (SI Appendix, Fig. S2G), may play an important role in this mechanism as NCoR interacts with Cdk5 (17).

Amorfrutins Prevent Formation of Insulin Resistance, Dyslipidemia, and Liver Steatosis Induced by HFD. To investigate the potential of the amorfrutins to prevent development of insulin resistance, C57BL/6 mice were fed for 15 w either a low-fat diet (LFD) or a HFD in the absence or the presence of rosiglitazone (HFD+R), or low-dose amorfrutin 1 (37 mg/kg/d) from the beginning of HFD feeding (HFD+A1), respectively. Amorfrutin 1 reduced the HFD-induced weight gain by 22% without affecting food intake (SI Appendix, Fig. S6 A and B), which indicates that early intervention by low-dose natural PPARγ agonists can reduce diet-induced weight gain and development of concomitant disorders such as insulin resistance. In the corresponding control experiment, synthetic rosiglitazone reduced HFD-induced weight gain even more strongly (SI Appendix, Fig. S8B), indicating that in general early intervention with PPARγ-modulating molecules may have different effects than late-stage treatment.

Consistently, presumably as an effect of both reduced weight gain and increased insulin sensitivity, preventive administration of amorfrutin 1 significantly improved glucose tolerance (22% decrease in insulin AUC) (Fig. 5A) and insulin sensitivity (21%...
increase in glucose AUC) (Fig. 5B). Additionally, this natural product substantially diminished the rise of plasma free fatty acids and triglycerides (SI Appendix, Fig. S8C). Preventive administration of amorfrutin 1 also maintained the integrity of the pancreas, as indicated by the plasma level of proinsulin that did not increase during 15 w of HFD-feeding (SI Appendix, Fig. S8D). Furthermore, amorfrutin 1 significantly reduced the increase of plasma concentrations of the adipose derived hormone leptin (Fig. 5C), which could have in part contributed to the improved metabolic profile.

Heavily overweight mice usually develop liver steatosis due to storage of fat in central organs (32). In stark contrast to rosiglitazone, amorfrutin 1 reduced HFD-induced accumulation of liver triglycerides by approximately 50% (Fig. 5D). To shed light on the potentially underlying mechanism of amorfrutin-based prevention of liver disorders in HFD mice, we determined gene expression profiles in liver tissue. As reported recently, accumulation of triglycerides in the liver is—although the exact molecular mechanism is still unclear—causally linked to decreased expression of transducin beta-like 1 (Tbl1), a transcriptional cofactor of PPARα, which is the master regulator of fatty acid oxidation (33). Consistent with previous results, Tbl1 expression negatively correlated with liver steatosis (SI Appendix, Fig. S9A), and HFD feeding of mice led to significant reduction in Tbl1 expression compared to LFD-fed animals (Fig. 5E). Treatment with amorfrutin 1, but not rosiglitazone, increased the gene expression of Tbl1 significantly (Fig. 5E). Rosiglitazone further hyper-activated for example Fabp4 expression by a factor of 55, accounting potentially in part for the increased lipid storage in the mouse liver (34) (Fig. 5F). In contrast, amorfrutin 1 rather induced the expression of genes responsible for fatty acid oxidation (Fig. 5F), likely at least in part via regulation of Tbl1. Furthermore direct interaction of amorfrutin 1 with the liver specific nuclear receptor PPARα and potentially additional modulation of PPARβ/δ pathways (Table 1, SI Appendix, Fig. S2A) may have contributed to the observed reduction of liver steatosis (Fig. 5D) (35).

Obesity is further characterized by the expression of inflammatory mediators and macrophage recruitment to different tissues (2, 36). In HFD-fed mice amorfrutin 1 decreased inflammation and macrophage accumulation in liver and visceral white adipose tissue (SI Appendix, Fig. S9 B–E). This anti-inflammatory effect was also reflected in reduced tumor necrosis factor α (TNFα) protein concentration in liver (Fig. 5G). Thus, amorfrutin treatment led to additional liver protective effects, including higher liver glycogen content, likely as a result of reduced insulin resistance in HFD-mice (SI Appendix, Fig. S9F) (37).

Potential Applications of the Amorfrutins. In summary, our results suggest that the plant-derived amorfrutins function as selective PPARγ modulators that induce beneficial changes in glucose metabolism and lipid profiles. In our mouse models, we further observed a reduction of inflammatory responses to metabolic stress. In contrast to many synthetic PPARγ agonists including the thiazolidinediones, amorfrutin treatment additionally had significant liver protective effects.

Much debate in the diabetes field has focused on the various side effects of the thiazolidinediones. For example, the widely applied strong PPARγ activator rosiglitazone did not only cause weight gain but also led to increased rates of cardiovascular disease in humans after long-term treatment, at least in part as a result of fluid or water retention. Consistently, in DIO mice rosiglitazone significantly decreased plasma protein concentration, suggesting increased fluid retention, whereas selective PPARγ agonist amorfrutin 1 did not change this physiological parameter compared to vehicle control (SI Appendix, Fig. S9G).

The fact that the amorfrutins are derived from edible plants may encourage more detailed study of their mode of action, as eventual regulatory approval for use in humans will be easier to obtain. PPARγ also plays central roles in inflammation (38) and aging processes (39). Thus it is possible that amorfrutin treatment could benefit other age-associated or inflammatory disorders, and cancer. Clearly, as for all potentially health-beneficial molecules, further in-depth studies including human studies will be required to assess the therapeutic potential of the amorfrutins. In general, further mechanistic studies on the PPARs will help to better describe the effects of structurally new PPAR-modulating compounds.

Our discovery of the highly antidiabetic legume-derived amorfrutins highlights the fascinating structural and biological properties of natural products, and suggests that dietary small molecules represent a largely unexplored resource for pharmaceutical and nutraceutical development. As ingredients of functional food or plant-based medicine for inhibiting insulin resistance and liver steatosis, dietary molecules such as the amorfrutins may have a great potential to be accepted by the consumers and patients as emerging alternatives to conventional treatment with synthetic drugs.

Materials and Methods

Compounds were purchased from the following sources: rosiglitazone (Cayman, Biozol), nTZDpa (Toeris, Biozol), pioglitazone (Sigma Aldrich), telmisartan, troglitazone, GW0742, GW7647 (all from Sigma Aldrich), amorfrutin 1 (NP-003520), amorfrutin 2 (NP-003521), amorfrutin 3 (NP-006430), amorfrutin 4 (NP-009525), natural product library (all available from Analytic Discovery) (SI Appendix, SI Methods). For screening of ligands we estab-
lished a mass spectrometry based heterogeneous binding assay that is parti-
cularly useful for rapid screening of natural product libraries containing
many autofluorescent compounds and new target proteins for which no
specific assay is available (SI Appendix, SI Methods). For in vivo testing, we
developed a method for the synthesis of multigram quantities of pure amor-
frutin 1 (SI Appendix, SI Methods). PPARY ligands were further characterized
using competitive binding assays (Lanthascreen, Invitrogen), coactivator rec-
ruitment assays (Lanthascreen, Invitrogen and Cerep Inc.) and reporter gene
assays (GeneBLazer, Invitrogen) (SI Appendix, SI Methods).

Effects of PPARY ligands were investigated in murine 3T3-L1 cells (ATCC,
LGc Promochem) and human primary adipocytes (Zen-Bio, BioCat). Gene
expression was measured with quantitative PCR (Applied Biosystems) and

Expression was measured with quantitative PCR (Applied Biosystems) and
2. Kahn SE, Hull RL, Utschneider KM (2006) Mechanisms linking obesity to insulin resis-
4. Huang TH, Kata BP, Razmovski V, Roufogalis BD (2005) Herbal or natural medicines as
modulators of peroxisome proliferator-activated receptors and related nuclear
tion of PPARGamma and alpha by punicit acid ameliorates glucose tolerance and
ameliorates glucose tolerance and obesity-related inflammation in db/ db mice
363:803–806.
gamma: too much of a good thing causes harm. EMBO Rep 5:142–147.
9:668–669.
4:315–322.
as insulin sensitizers: from the discovery to recent progress. Curr Top Med Chem
8:1483–1507.
16. Li P, et al. (2011) Adipoocyte NCoR knockout decreases PPARGamma phosphorylation
in complex with AZ 242; ligand selectivity and agonist activation in the PPAR family.
21. Schupp M, et al. (2005) Molecular characterization of new selective peroxisome pro-
liferator-activated receptor gamma modulators with angiogenin receptor blocking
22. Fischer JJ, et al. (2011) Dasatinib, imatinib and staurosporine capture compounds—
Complementary tools for the profiling of kinases by Capture Compound Mass
23. Ong SE, et al. (2009) Identifying the proteins to which small-molecule probes and
receptors gamma and alpha on food intake and energy homeostasis. Diabetes
52:2249–2259.
syndrome. JAMA 285:377–381.
29. Torreggiani L, et al. (2010) PPAR-alpha is involved in adipogenesis and in the regula-
syndrome. JAMA 285:377–381.
33. Kuda O, et al. (2009) Prominent role of liver in elevated plasma palmitoleate levels in