Pleiotropic responses to interferon-gamma in humans

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Chapter 6

Interferon-gamma influences glucose and fat metabolism and restores monocyte HLA-DR expression in surgical patients

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Submitted for publication
Abstract

Tissue injury is associated with decreased cellular immunity and enhanced metabolism. The first, immunosuppression, is thought to be counteracted by recombinant human (rh) interferon-gamma (IFN-γ) administration since IFN-γ is a well known upregulator of human leukocyte antigen-DR (HLA-DR) expression. The latter however, hypermetabolism, could be enhanced after rhIFN-γ administration since inflammatory cytokines are thought to play an important role in the pathophysiology of the metabolic response to stress. In healthy humans rhIFN-γ enhanced HLA-DR expression without important effects on glucose and fat metabolism. In the present study we evaluated whether rhIFN-γ also lacks harmful side effects on the metabolic and endocrine pathways while maintaining its beneficial effects on the immune system under conditions in which the host inflammatory response system is activated. Therefore we studied in thirteen patients, scheduled for major surgery (pylorus-preserving pancreaticoduodenectomy) HLA-DR expression on peripheral blood monocytes prior to surgery and postoperatively randomized the patients into an intervention and placebo group. Subsequently, we evaluated the effects of a single dose of rhIFN-γ (Immukine, 100μg/m², s.c.) vs. saline, on short-term monocyte activation, glucose and lipid metabolism and glucose- and lipid regulatory hormones. The rate of appearance of glucose and glycerol was determined by infusion of d2-glucose and d5-glycerol respectively. HLA-DR expression on monocytes was restored 24 hours after IFN-γ administration but stayed low in the placebo treated patients. IFN-γ decreased glucose production and increased lipolysis without effects on plasma glucose levels and only a slight increase in plasma free fatty acids. There was no effect of IFN-γ on plasma concentrations of cortisol, adrenocorticotropic hormone, growth hormone, insulin, c-peptide, glucagon and epinephrine, but an increase in plasma norepinephrine levels. We conclude that IFN-γ exerts a favourable effect on cell-mediated immunity in patients after major surgery without clinically important, endocrine and metabolic side effects.
**Introduction**

Tissue injury is associated with decreased expression of human leukocyte antigen-DR (HLA-DR) on monocytes (1-6). Low levels of HLA-DR on monocytes are an ominous prognostic factor in the recovery of surgical patients, since reduced HLA-DR expression (or a defective antigen presentation or cellular immunity) is correlated with increased postoperative complications and mortality (7). Downregulation of HLA-DR expression on monocytes may partly be related to a surgically directed shift of the T-helper 1 (Th1)/Th2 balance toward a dominating Th2 type immune response (8). Compared to preoperative production, post-operatively stimulated peripheral blood mononuclear cells (PBMC) produce significantly more Th2 cytokine interleukin (IL)-4 than its Th1 counterpart interferon-gamma (IFN-γ) (8,9).

Administration of IFN-γ enhances the expression of HLA-DR on monocytes in vitro and in vivo(10). Several small to intermediate-sized clinical trials have addressed the effect of IFN-γ on HLA-DR expression and clinical recovery after sepsis (11) and severe injury (12-15). These studies suggest that administration of IFN-γ to surgical patients could have positive effects on postoperative recovery and prevention of complications, although the data are not unambiguous at present (16,17).

In addition to the reduction in cellular immunity, another –potentially harmful- side effect of surgical interventions is the metabolic response to stress (18,19). This response, which is characterised by catabolic reactions like increases in glucose production, lipolysis and protein turnover, is mediated by the interaction between hormones, inflammatory mediators and the central nervous system (20,21). Administration of inflammatory mediators like tumor necrosis factor (TNF)-α, IFN-α, IL-2 or IL-6 to humans, mimics the catabolic changes observed after tissue injury, with specific effects for each individual cytokine (22-24). In a previous study, we administered rhIFN-γ (100μg/m², s.c.) to healthy subjects in a saline controlled cross-over study with measurements of HLA-DR expression on monocytes and endocrine and metabolic parameters (25). IFN-γ induced a profound increase in HLA-DR expression on monocytes, whereas, in contrast to other cytokines tested in a comparable setting, IFN-γ exerted surprisingly small endocrine and metabolic effect (25).
Considering these observations, IFN-γ seems to be an ideal cytokine to improve the cellular immune function in surgical patients without an additional negative influence on an activated metabolic and endocrine system. However, our observations in healthy subjects do not exclude a possible deviated endocrine and/or metabolic reaction to IFN-γ in surgical patients. For instance, IFN-γ may act synergistically with the many inflammatory response proteins that abundantly circulate in post-operative patients (26-28). The question arises therefore, whether IFN-γ also lacks harmful side effects on the metabolic and endocrine pathways under conditions in which the host inflammatory response system is activated, while maintaining its beneficial effects on the immune system. To our knowledge, no studies have been published in which acute metabolic, endocrine and immunological effects of IFN-γ administration are studied simultaneously in a homogeneous surgical patient population.

In patients, scheduled for major surgery (pylorus-preserving pancreaticoduodenectomy) we evaluated HLA-DR expression on peripheral blood monocytes prior to surgery. Postoperatively, patients were randomised into an intervention (IFN-γ) and placebo (control) group. Subsequently, we evaluated the effects of a single dose of recombinant human (rh)IFN-γ (Immukine, 100μg/m², s.c.) vs. saline, on short-term monocyte activation and metabolic and endocrine parameters.

**Subjects and Methods**

**Subjects**
Between December 1998 and December 1999 twenty-four patients were included in the study. All patients were scheduled for elective pylorus-preserving pancreaticoduodenectomy (pppd) aimed at curative treatment of a suspicious tumor in the pancreatic head, papilla of Vater, distal bile duct or duodenum. Exclusion criteria were: a) *any* other diseases than the currently treated disorder (including diabetes mellitus associated with the primary disease); b) jaundice at hospital admission (bilirubin levels >40 μmol/L, preoperative biliary drainage was accepted); c) fever in the two weeks prior to hospital admission; d) any medication at admission to the hospital (except for paracetamol, pancreatic enzyme supplement or sleep medication); e) irresectability as a peroperative finding, and
therefore deviation of the intended pppd procedure towards a bypass procedure; f) clinical instability or evidence of infection on the day of the study (the second post-operative day).

All patients gave written informed consent, in accordance with the Helsinki Declaration of Human Rights. The study was approved by the Research Committee and the Medical Ethical Committee of the Academic Medical Center, Amsterdam.

**Study design**

One day prior to the surgical procedure, blood was sampled for measurement of HLA-DR expression on monocytes and routine biochemical and haematological measurements (including bilirubin). From 06.00 P.M. at the preoperative day till 05.00 P.M. at the second postoperative day, patients were only permitted to drink water in accordance with the protocol for this surgical procedure.

During surgical intervention, patients were anesthesized using isoflurane and sufentanyll supplemented with drugs provided to the discretion of the anaesthesiologist. Moreover, a high thoracic epidural catheter was inserted through which marcaine was administered. On the first post-operative day, patients were transferred from the recovery room to the general surgical ward. From 0.00 A.M. at the first postoperative day until 5.00 P.M at the second postoperative day, intravenous infusion fluids were limited to saline. Oral food supply or usage of the feeding jejunostomy was only allowed from 06.00 P.M. the second postoperative day onwards according to the treatment protocol. Analgesics were prescribed according the standard hospital protocol (paracetamol, morphine and epidural marcaine). Additionally, all patients received Fraxiparin® (Sanofi, Maassluis, The Netherlands) and Sandostatin® (Novartis Pharma LtD. Basel, Swiss).

At the second post-operative day patients were randomized to the IFN-γ or control group by balanced assignment. At 6.45 h A.M., a catheter was placed retrogradely into an antecubital vein for sampling of arterialized venous blood, which was realised by placement of the forearm in a thermoregulated (65°C) Plexiglas box during 20 minutes before blood was sampled. Another catheter, situated already in the contralateral hand vein or a central venous catheter -if available- was used for infusion of stable isotope tracers. Both catheters were kept patent by infusion of NaCl 0.65% (30 ml/h). At 7.00 h A.M. blood was sampled for determination of background isotope enrichment. Subsequently, a primed (17.6 μmol/kg), continuous (0.22 μmol/kg/min) infusion of [6,6-²H₂]glucose (CIL, Andover, MA, USA) and a primed
(1.5 μmol/kg), continuous (0.1 μmol/kg/min) infusion of \([1,1,2,3,3-^{2}H_{5}]\)-glycerol (CIL, Andover, MA, USA) were started and continued until the end of the first study day (t = 8 h). At \(t = -10\), \(-5\) min. and just before rhIFN-γ or saline administration, blood samples for determination of isotope enrichment of \([6,6-^{2}H_{2}]\)glucose and \([1,1,2,3,3-^{2}H_{5}]\)glycerol were drawn. Blood samples for baseline values of plasma hormones, substrates and cytokines and HLA-DR expression on monocytes were drawn just before rhIFN-γ or saline solution was administered. At 09.00 A.M. (\(t=0\) h), rhIFN-γ (100 μg/m², Immukine, Boehringer Ingelheim GmbH, Ingelheim/Rhein, Germany) or a comparable volume of saline solution was injected subcutaneously in the upper leg. At 1, 2, 4, 6, and 8 h after injection of rhIFN-γ or saline, blood was drawn for the measurement of isotope enrichment, hormone, substrate and cytokine concentrations. Twenty-four and 48 hours after the injection of rhIFN-γ or saline blood was drawn for determination of plasma cytokine levels. Blood samples taken at 8 and 24 hours after administration of rhIFN-γ were also analysed for HLA-DR expression on monocytes. Blood pressure (Riva Rocci method, brachial artery), pulse rate (palpation of radial artery) and oral temperature (Terumo Dig. Clin. Thermometer C11, Terumo Corp., Tokyo, Japan) were recorded on the first study day at the bloodsampling timepoints. Body composition was measured at the seventh post operative day by Bio Impedance Analysis (BIA 109 Bio Impedance Analyzer, Akern, Florence, Italy).

**Assays**
All measurements of each individual subject were performed in the same run and tested in duplicate, with the exception of flow cytometric analysis, which was performed immediately after blood sampling.
Plasma glucose concentrations and enrichment were determined according to Reinhauer et al.(29), using xylose as internal standard. The gas chromatography column used was a J&W DB-17 capillary column (30 x 0.25 mm, d = 0.25 μm) (J&W, Folsom, CA) on an HP 6890 Series gas chromatograph coupled to an HP 5973 mass selective detector (Hewlett Packard, Palo Alto, CA). Mass spectra were recorded at m/z 187 for glucose and m/z 189 for 6,6-\(^{2}H_{2}\)-glucose. The internal standard was monitored at m/z 145. Plasma glycerol concentrations and enrichment were determined as described previously(30).
Metabolic and immunological effects of IFN-γ in surgical patients

Free fatty acid (FFA) concentrations in plasma were determined using the NEFA C kit (code No 994-75409 E) from Wako Chemicals GmbH (Neuss, Germany). Plasma insulin concentration was measured by RIA (Insulin RIA 100, Pharmacia Diagnostic AB, Uppsala, Sweden; intra-assay coefficient of variation (CV) 3-5%, inter-assay CV 6-9%), C-peptide by RIA (RIA-coat c-peptide, Byk-Sangtec Diagnostics GmbH & Co KG, Dietzenbach, Germany; intra-assay CV 4-6%, inter-assay CV 6-8%). Glucagon was determined by RIA (Linco Research, St Charles, MO; detection limit 15 ng/L, intra-assay CV 3-5%, inter-assay CV 9-13%), IGF-1 by IRMA after a modified acid-ethanol extraction procedure (DSL, Inc. Webster, Texas; detection limit 5 nmol/L, intra-assay CV 2-4%, inter-assay CV 3-8%). Cortisol was measured using a luminescence enzyme immunoassay, Immulite (Cortisol, Diagnostic Products Corporation, LA, intra-assay CV 5.8%, inter-assay CV 7.0%), adrenocorticotrophic hormone (ACTH) by ILM A (Immuno Luminometric Assay)(Nichols Institute, Los Angeles, CA; intra- and inter-assay CV 4.3 and 5.4 %, resp.), growth hormone (GH) by ILMA (Nichols Institute, Los Angeles, CA; detection limit 1 mU/L, intra- and inter-assay CV 7.3% and 9.6%, resp). Catecholamines were measured by in-house HPLC method. Norepinephrine (inter- and intra-assay CV 13 and 6 %, resp.) and epinephrine (inter- and intra-assay CV 14 and 7 %, resp.) were selectively isolated by liquid-liquid extraction and derivatized to fluorescent components with 1,2-diphenylethlyenediamine. The fluorescent derivatives were separated by reversed phase liquid chromatography and detected by fluorescence detection (31,32)

IFN-γ was measured using an in-house sandwich ELISA with a detection limit of 31 pg/ml with monoclonal antibody (mAb) MD2 as capture and biotinylated MD1 as detecting mAb, with a detection limit of 31 pg/ml, IL-6, and TNF-α were determined by ELISA (CLB, Amsterdam, The Netherlands), both with a detection limit 2 pg/ml. IL-10 was also measured by ELISA (Schering-Plough Research Institute, Kenilworth, NJ), with a detection limit of 20 pg/ml. Monocyte HLA-DR expression was measured using flow cytometry as described previously(25). Cells were incubated with anti-HLA-DR monoclonal antibodies (mAbs) directly labelled with fluorescein isothiocyanate (FITC, Becton Dickinson (BD), San Jose, CA). Irrelevant mouse mAbs directly labelled with FITC (BD) were used as control for background staining. Data acquisition was performed on a FACScan flow cytometer (BD). Monocytes were gated by forward and side scatter parameters.
Calculations and statistics
All data are presented as the mean ± SEM. The endogenous glucose and glycerol production ($R_a$) and glucose- and glycerol disposal ($R_d$) were calculated using Steele's equation for non-steady state conditions adapted for stable isotopes:

$$R_a(t) = \frac{I}{PCT_p(t)} - \frac{pVG(t)[dPCT_p(t)/dt]}{PCT_p(t)}$$

$$R_d(t) = \frac{I}{PCT_p(t)} - \frac{pVG(t)[dPCT_p(t)/dt]}{PCT_p(t)} - \frac{dG(t)}{dt}$$

where $I$ is the constant tracer infusion rate (mg·kg⁻¹·min⁻¹), $PCT_p(t)$ is the percent enrichment in plasma glucose or glycerol taken as the average of two consecutive samples, $p$ is the pool fraction, $V$ is the distribution volume of glucose (165 ml/kg) or glycerol (235 ml/kg), $G(t)$ is the plasma glucose or glycerol concentration taken as the average of two consecutive samples, $dPCT_p(t)/dt$ is the rate of change of percent enrichment in plasma (min⁻¹). $R_a$ glycerol levels were corrected for total body fat mass as measured by Bio Impedance Analysis.

Data were analysed by analysis of variance for randomised block design and, to test data between the two study arms at individual time points, a paired t-test for two unrelated samples or a Mann-Whitney test was used. A p-value of < 0.05 was considered to represent statistical significance. Preoperative data represent patients that finished the total protocol.

Results

Patient characteristics
Initially, twenty-four patients were included in the study. Postoperatively, ten patients were excluded from the study, because the tumor was irresectable with concomitant deviation of the pppd procedure. One other patient was excluded on
Metabolic and immunological effects of IFN-γ in surgical patients

### TABLE 1. Patient Characteristics

<table>
<thead>
<tr>
<th>IFN-gamma</th>
<th>placebo</th>
</tr>
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<tr>
<td>sex</td>
<td>sex</td>
</tr>
<tr>
<td>(male/female)</td>
<td>(male/female)</td>
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<tr>
<td>7</td>
<td>7</td>
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<tr>
<td>mean</td>
<td>mean</td>
</tr>
<tr>
<td>SE</td>
<td>SE</td>
</tr>
</tbody>
</table>

Data are expressed as individual measures and mean ± SE

No significant differences were detected between the groups

† : counted from the first postoperative day

the second post-operative day before IFN-γ/saline was administered, because of cardiac and respiratory instability. Thirteen other patients continued the study on the second postoperative day and were randomly assigned to the placebo or intervention group. Seven patients received 100 μg/m² rhIFN-γ subcutaneously, whereas six other patients received a similar volume of isotonic saline. Clinical characteristics of these patients are given in table 1.

**Clinical effects of IFN-γ**

In both study arms the incidence of clinical symptoms as chills, nausea and headache was comparable. There were no differences between the two study arms...
in baseline body temperature and there were no effects in time in the control arm of the study. IFN-γ caused an increase in body temperature compared to controls with a maximum level after 8 hours (baseline: IFN-γ 37.0±0.2 °C, controls 37.1±0.2 °C; t=8h: IFN-γ 38.0±0.2 °C, controls 37.4 °C, p<0.001). During hospital admission in each group one patient suffered from an infectious complication, but recovered after treatment with antibiotics.

**Plasma cytokine levels**

Preoperative and baseline IFN-γ levels fluctuated around the lower limit of detection of our assay (31 pg/ml). During the control study, no changes from baseline levels were detected. After injection of IFN-γ, IFN-γ serum levels gradually increased to a peak level of 118±12 pg/ml (p<0.007 vs. controls). Twenty-four hours after IFN-γ administration, IFN-γ levels were back to baseline. Preoperative and postoperative levels of TNF-α and IL-10 were below the detection limit of the assays (2 pg/ml). There were no effects in time on TNF-α and IL-10 levels during the control and intervention study. Preoperative IL-6 levels were around the lower limit of detection of the assay (2 pg/ml). Postoperatively, IL-6 levels were high in several subjects, predominantly in the control group (median and 25-75 percentiles at baseline; 59(50-132) pg/ml for IFN-γ vs. 85(43-

![Figure 1](image-url)  
**Figure 1** Number of HLA-DR positive monocytes, preoperative (preO), postoperative (postO) and 8 and 24 hours after the administration of 100 µg/m² rhIFN-γ (closed bars, n=7) or saline (open bars, n=6). Data are expressed as mean ± SE. ¶ p<0.05, ★ p=0.004 vs. corresponding value in control patients.
240) pg/ml for controls, not significantly different (NS) between groups, and decreased slightly during the intervention study (p<0.05), whereas no effect in time was measured during the control study and no differences were detected between the two study arms.

Markers of monocyte activation (fig. 1).

**HLA-DR:** Preoperatively, mean HLA-DR expression on peripheral blood monocytes was 92±2%. On the second postoperative day, just prior to injection of IFN-γ, monocyte HLA-DR expression was approximately halved, to 50±3 and 40±5 % for the intervention and control study respectively (intervention and control group preoperative vs. postoperative t=0 p<0.0001; NS between groups). In the control group, the decline in HLA-DR expression continued during the next 24 hours, to a mean level of expression of 36±10 %. In contrast, in the intervention group, IFN-γ restored the HLA-DR expression, which already started at t=8h after injection (p<0.05 vs. controls) and was completed after 24 hours (93±1 %, p=0.004 vs. controls).

**TABLE 2.** Plasma hormone concentrations at baseline and after the administration of rhlIFN-γ (n=7) or saline (controls, n=6) to postoperative patients

<table>
<thead>
<tr>
<th>time (h)</th>
<th>ACTH (ng/l)</th>
<th>cortisol (nmol/l)</th>
<th>epinephrine (nmol/l)</th>
<th>norepinephrine (nmol/l)</th>
<th>insulin (pmol/l)</th>
<th>glucagon (nmol/l)</th>
</tr>
</thead>
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<tr>
<td>IFN-gamma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>14 ± 4</td>
<td>496 ± 65</td>
<td>0.40 ± 0.09</td>
<td>2.6 ± 0.8</td>
<td>42 ± 11</td>
<td>79 ± 13</td>
</tr>
<tr>
<td>1</td>
<td>50 ± 23</td>
<td>665 ± 78</td>
<td>0.38 ± 0.11</td>
<td>3.7 ± 0.9</td>
<td>35 ± 9</td>
<td>75 ± 9</td>
</tr>
<tr>
<td>2</td>
<td>31 ± 14</td>
<td>707 ± 91</td>
<td>0.43 ± 0.13</td>
<td>3.9 ± 0.7</td>
<td>36 ± 9</td>
<td>74 ± 11</td>
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<td>4</td>
<td>19 ± 7</td>
<td>554 ± 57</td>
<td>0.36 ± 0.13</td>
<td>5.3 ± 1.7</td>
<td>38 ± 8</td>
<td>79 ± 11</td>
</tr>
<tr>
<td>6</td>
<td>26 ± 8</td>
<td>710 ± 64</td>
<td>0.37 ± 0.11</td>
<td>4.9 ± 0.6</td>
<td>37 ± 8</td>
<td>83 ± 9</td>
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<tr>
<td>8</td>
<td>14 ± 4</td>
<td>577 ± 46</td>
<td>0.38 ± 0.16</td>
<td>3.3 ± 0.6</td>
<td>40 ± 10</td>
<td>82 ± 9</td>
</tr>
<tr>
<td>placebo</td>
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<td>ACTH (ng/l)</td>
<td>cortisol (nmol/l)</td>
<td>epinephrine (nmol/l)</td>
<td>norepinephrine (nmol/l)</td>
<td>insulin (pmol/l)</td>
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<td>0</td>
<td>16 ± 7</td>
<td>647 ± 152</td>
<td>0.19 ± 0.08</td>
<td>1.7 ± 0.3</td>
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<td>710 ± 175</td>
<td>0.15 ± 0.06</td>
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<td>77 ± 9</td>
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<td>20 ± 10</td>
<td>857 ± 226</td>
<td>0.13 ± 0.06</td>
<td>2.3 ± 0.5</td>
<td>31 ± 4</td>
<td>71 ± 6</td>
</tr>
<tr>
<td>4</td>
<td>8 ± 2</td>
<td>657 ± 151</td>
<td>0.16 ± 0.08</td>
<td>2.0 ± 0.5</td>
<td>30 ± 4</td>
<td>64 ± 5</td>
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<tr>
<td>6</td>
<td>29 ± 19</td>
<td>628 ± 107</td>
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<td>2.1 ± 0.5</td>
<td>30 ± 8</td>
<td>63 ± 4</td>
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<tr>
<td>8</td>
<td>12 ± 3</td>
<td>580 ± 76</td>
<td>0.18 ± 0.09</td>
<td>2.1 ± 0.4</td>
<td>36 ± 7</td>
<td>69 ± 5</td>
</tr>
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</table>

Data are expressed as mean ± SE

\* \* p<0.02 vs controls
Hormones (table 2.)
There were no differences between the two groups in baseline hormone levels. In the IFN-γ and control group, there were no changes from baseline levels during the study. IFN-γ did not have an effect on plasma hormones as compared to controls, except for an increase in norepinephrine levels at t=6 hours (p<0.02 vs. controls).

Substrates of energy metabolism and glucose and glycerol kinetics (fig. 2, 3 and table 3.)
Glucose: Baseline values did not differ between the two studies. There was no effect of IFN-γ or time on plasma glucose levels during the study. IFN-γ induced a significant greater decline of ~30% in $R_a$ of glucose as compared to controls in the first hour after administration (decrease in the first hour, IFN-γ vs. control: 3.5±0.4 vs. 2.7±0.3 μg/kg/min, p<0.05). Subsequently, in both groups the decline in $R_a$ glucose was parallel (NS between groups). There was no effect of IFN-γ or placebo on glucose disposal.
Fat: Baseline values of plasma FFA, plasma glycerol and $R_a$ glycerol did not differ between the two studies (Table 3). There was no effect of IFN-γ on plasma glycerol levels. IFN-γ induced a significant increase in plasma FFA levels (p<0.05 vs. controls). In parallel, IFN-γ increased $R_a$ glycerol significantly as compared to controls (p<0.05 respectively 0.02 vs. controls).
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Figure 3  Effects of IFN-γ on fat metabolism: Plasma FFA concentration and rate of appearance (Ra) of glycerol after 100 μg/m² rhIFN-γ (closed circles, n=7) or saline (open circles, n=6) administration to postoperative patients. Data are expressed as mean ± SE. *p<0.05 vs. corresponding value in control patients. Baseline values are presented in Table 3.

<table>
<thead>
<tr>
<th></th>
<th>FFA (mmol/L)</th>
<th>Glycerol (μmol/L)</th>
<th>Ra glycerol (μmol/kg/min)</th>
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<tbody>
<tr>
<td>IFN-gamma</td>
<td>0.58 ± 0.06</td>
<td>82 ± 12</td>
<td>1.00 ± 0.16</td>
</tr>
<tr>
<td>Placebo</td>
<td>0.74 ± 0.08</td>
<td>99 ± 6</td>
<td>1.14 ± 0.16</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE
No significant differences were detected between the groups

Discussion

In the present study, we evaluated in postoperative patients, the short term effects of IFN-γ on immunological, endocrine and metabolic parameters. The surgical intervention induced a reduction of monocyte HLA-DR expression, that was completely restored by IFN-γ. IFN-γ induced an initial decrease in endogenous glucose production as compared to controls without effect on plasma glucose levels and glucoregulatory hormones concentrations, except for a small increase in plasma norepinephrine levels. Moreover, IFN-γ induced a modest increase in lipolysis, reflected in increases in plasma FFA levels and Ra glycerol. Thus, in contrast to the results obtained in our healthy volunteers, in surgical patients IFN-γ had specific although modest metabolic effects (25). Finally, a single dose of IFN-γ (100 μg/m²) did not induce significant clinical side effects in these postoperative patients.

The present study is the first study in which preoperative and postoperative levels and the effects of IFN-γ administration on HLA-DR expression on monocytes were measured in one combined study. Enhanced levels of HLA-DR
expression on monocytes after IFN-\(\gamma\) injection compared to controls have been described previously in postoperative patients with colon cancer, severely injured patients and in septic patients (11, 33, 34). In contrast to the present study, in none of these studies measurements were performed prior to (elective) trauma or the disease. In the patients with colon cancer, IFN-\(\gamma\) treatment (200 \(\mu\)g/d) was started three to four weeks after curative elective surgery and HLA-DR expression was measured after one month of treatment (33). In these patients HLA-DR expression increased from \(\sim\)88% at baseline to \(\sim\)97% after 1 month of treatment, whereas postoperative HLA-DR expression stayed low in the control group during the total follow-up period of 12 weeks. In trauma patients, receiving 100 \(\mu\)g/d, HLA-DR levels on monocytes prior to treatment with IFN-\(\gamma\) were 57% and increased to 76% after two days of treatment with IFN-\(\gamma\) (34). In septic patients, HLA-DR expression on monocytes prior to treatment was 27% and IFN-\(\gamma\) (100\(\mu\)g/d) increased HLA-DR expression to 62% within 24 h (11). Our patients presented with HLA-DR expression levels after surgery that were immediately reduced (50% 2nd day postoperative), but reached higher levels of expression 24 hours post IFN-\(\gamma\) (93%). In all these studies including our own, IFN-\(\gamma\) clearly increased HLA-DR expression, reflecting a significant recovery of monocyte function. This effect of exogenous IFN-\(\gamma\) on monocytes in postoperative patients was obtained despite significantly lower peak levels in plasma of IFN-\(\gamma\) compared to our healthy subjects (25). Since the dose of IFN-\(\gamma\) was equal in both studies, it must be concluded that the clearance of IFN-\(\gamma\) is increased after surgery, a finding that has also been described for other mediators like insulin (35).

Cytokines are thought to play an important role in the metabolic response to injury (18). Administration of cytokines like TNF-\(\alpha\), IL-6 and IFN-\(\alpha\) in humans induces an hypermetabolic state, which is reflected by cytokine-specific elevations in resting energy expenditure and increases in glucose and glycerol turnover (22-24). IFN-\(\gamma\), commonly marked as an important pro-inflammatory cytokine, was imputed a comparable effect on hormones and metabolism (36). In healthy humans, however, IFN-\(\gamma\) only induced a limited increase in REE and did not affect glucose metabolism (25). Surprisingly, in the present study in postoperative patients, IFN-\(\gamma\) induced an early depression of the endogenous glucose production compared to controls. Until now, IFN-\(\gamma\) is the only cytokine known with such a depressant effect on glucose production in humans in vivo. This phenomenon was
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not a result of alterations in glucoregulatory hormones. The small increase in norepinephrine levels as measured after IFN-γ, would have induced an increase in glucose production (37). The influence of IFN-γ on the Rₐ glucose reflects therefore most likely a direct effect of IFN-γ on hepatic glucose production, potentially mediated by NO. Hepatic glucose production is regulated by the interplay of several mechanisms, amongst which glucoregulatory hormones, substrate concentrations and paracrine mediators produced by Kupffer cells. In the liver, there is intensive interaction between Kupffer cells and hepatocytes. In vitro data show that products of Kupffer cells, like prostaglandins and nitric oxide (NO) influence hepatic glucose production (38). In humans, stimulation of prostaglandin synthesis has inhibitory effects on human hepatic glucose production in the absence of changes in plasma insulin and glucagon levels (39), whereas, conversely, inhibition of prostaglandin synthesis by indomethacin increases basal glucose production (40). A role for NO as a paracrine factor, that could mediate the effect of IFN-γ on glucose production, is derived from in vitro studies in which NO inhibits glucose production in vitro (41,42). IFN-γ induced production of NO by Kupffer cells in vitro is unambiguously described, whereas data on prostaglandin release by monocytes and Kupffer cells are less unequivocal (43-45). Measurement of NO concentrations in blood meets technical difficulties since NO is a very unstable molecule. Nitrate, a stable degradation product of NO, however, can be measured in plasma. In the present study, we could not find an effect of IFN-γ on serum nitrate levels (data not shown). This observation does not exclude locally increases in NO levels, which are not necessarily reflected by changes in plasma concentrations. IFN-γ is a potent activator of Kupffer cells, which are, like blood monocytes, derived from the myeloid lineage. The acute effect (first study hour) of IFN-γ may be explained from the anatomy: Kupffer cells reside on the luminal side on top of the endothelial cells in the liver sinusoid and will therefore be exposed to relatively high concentrations of circulating IFN-γ.

In the present study, an increase in lipolysis was paralleled by increased levels of norepinephrine, a major stimulator of lipolysis. A direct effect of IFN-γ on lipolysis is also a possibility, since IFN-γ can increase lipolysis in cultured adipocytes, although relatively high dosages are necessary (46). Our observation in surgical patients is in contrast to our data obtained in healthy volunteers, in which
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IFN-γ did not affect FFA plasma levels (25) or \( R_a \) glycerol (de Metz, unpublished data).

Previous human studies reported the effects of IFN-γ on HPA-axis activation. Increased cortisol release was unanimously found in these studies, whereas data on modulation of ACTH release are contradictory (25,47-49). The studies in which no ACTH increase was measured preceding a cortisol peak suggest the possibility of an ACTH-independent effect of IFN-γ on the adrenal glands (47,48). In the present study no effects of IFN-γ on ACTH or cortisol levels could be detected. High baseline cortisol levels may explain the lack of IFN-γ effect on cortisol levels in postoperative patients. The absence of an effect on ACTH in the present study may be due to the absence of an effect of IFN-γ on the mediator which could account for ACTH release in the previous studies: plasma IL-6, a known stimulator of ACTH release (24).

It can not be deduced from our study to what extent IFN-γ administration will result in improvement in clinical outcome. In three randomised trials IFN-γ administration to severely injured (trauma/burn) or sepsis patients did not result in definitive improvement in clinical relevant endpoints. Nevertheless, there is reason to believe that high risk patients might benefit from adjuvant IFN-γ therapy (12-14,17).

We conclude that IFN-γ exerts a favourable effect on cell-mediated immunity in patients after major surgery. Moreover, IFN-γ does not have considerable clinical, endocrine or metabolic side effects, although attention has to be paid to its moderate inhibitory effect on glucose production, especially in patients with septic shock, since hypoglycaemia is sometimes found in this syndrome (50).

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